Taxonomy and Molecular Phylogeny of *Hemidactylus*in the mainland of Yemen

(Class: Reptilia, Order: Squamata, Family: Gekkonidae)

Von der Fakultät für Lebenswissenschaften
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Dedication

This thesis is dedicated to my country 'Yemen' which is struggling to develop and revive.

Salem

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Introduction

Systematics is a unique field in natural sciences which has inspired many researchers to conduct studies since the earlier times and continues to gain more importance in modern times after the discovery of DNA techniques. This field of biology deals with the diversity of organisms and their relationships. However, this science is strictly historical rather than experimental since systematists cannot repeat controlled experiments for what will happen again in the evolution of living organisms as astronomers cannot in the evolution of stars. Consequently, taxonomists face problems when they consider the suitable epistemology in the history of living organisms. In point of fact, inaccurate work in natural sciences such as physics, chemistry and other natural sciences can be buried and ignored, whereas this is not true in systematics. Unfortunately, the terrible systematist is immoral since the incorrect definition remains a synonym for the taxon and attached to the name of the scientist. Thus, bad work does not die with the author, and everybody wants to be known as good workers for generations ahead (Wenzel 2002).

The important aspects in the classification of organisms are the characters. Characters in living organisms are divided into morphological and molecular traits. The genetic constituent of an organism is called genotype which refers to the particular alleles present in an organism at all loci that affect the trait. In contrast, the physical expression of a genotype is called the phenotype. The distinction between the genotype and the phenotype is particularly important in cases in which the environment can affect the trait (Hartl and Clark 1997).

In recent times, after the discovery of DNA and the advanced methods that depend on DNA it has become of great significance to use these modern techniques associated with classical taxonomy which relies on measurements and description of morphological characters. Recently, DNA techniques, specifically sequencing, have been introduced into systematics of lizard and other living organisms.

Every living organism has DNA (Deoxyribonucleic acid) in the structure of its cells. All detail of a living being is coded in this structure. DNA is a chemical structure that forms chromosomes. A piece of a chromosome that dictates a particular trait is called a gene. The position of a gene along a chromosome is called the locus of the gene. DNA is working as a data bank, all information of living things stored in this data bank. Structurally, the DNA molecule is a double-stranded helix, with the sugar-phosphate backbone of the antiparallel polynucleotide strands on the outside of the helix. Holding the two strands together are pairs of nitrogenous bases (also called nucleotides) attached to each other by hydrogen bonds. In a double helix, the strands go opposite ways. A nitrogenous base is one of four chemicals (adenine (A), guanine (G), cytosine (C) and thymine (T)). Each base is only bond with one other base, as follows: Adenine will only bond with thymine, and guanine will only bond with cytosine. The DNA molecule can be very long, for example, the DNA in the bacterium *E. coli* is about 4.7 million base pairs, and the largest chromosome in the fruit fly *Drosophila melanogaster* is about 65 million base pairs, however, in human it is about 230 million base pairs. DNA strands are read in a particular direction, from the top (called the 5' or "five prime" end) to the bottom (called the 3' or "three prime" end). The term 5' and 3' refer to the polarity of the strands. The chemical structure of the DNA of organisms is the same. However, the only difference

between them is the order of the base pairs (Hartl and Clark 1997, Campbell et al., 2008). Using these sequences, every organism could be identified solely by the sequence of the base pairs. Our understanding of how species evolve and diverge advanced after many studies proving genetic differentiation in association with genealogical lineages (Avise et al., 1987). Molecular methods are well known to be the best techniques to describe the genetic structure of populations.

Studying the mitochondrial DNA is very important to build the phylogenetic tree of living organisms, as it is the most useful molecule to infer the phylogeography at the level of conspecific populations and closely related species (Walker and Avise 1998). The mitochondrial DNA of vertebrates is a closed circular molecule. The mtDNA size is smaller compared to genomic DNA, and it sequence evolution rate is generally high; this high rate is the product of both a high mutation rate and a high mutation fixation rate. The high mutation rate results in part from the mtDNAs lack of protective histones, inefficient DNA repair systems, and continuous exposure to mutagenic effects of the oxygen radicals. The high mutation fixation rate is due to the efficient intracellular sorting of mutant molecules in the female germ line and the rapid genetic drift of mtDNAs in the general population (Brown, et al., 1979; Wallace, 1994; Hartl, 1998).

The main reason to use mtDNA in molecular studies since it is present in a large number of copies per cell, making possible the amplification of any particular gene by means of Polymerase Chain Reaction (PCR), even if a small amount of sample is taken. For example, the cytochrome *b* gene studied has been shown to possess enough variability among species of the genus *Hemidactylus* making the process of species identification easier. The template DNA strand is now copied with high

fidelity, eliminating the nonspecific products that had plagued earlier attempts at amplification. The field has been dominated by the use of mitochondrial DNA to determine phylogenetic relationships among animal populations, subspecies and species, which may be then corresponded with their geographical distribution (Hewitt 2001).

The discovery of PCR (polymerase chain reaction) contributed to the advance of molecular biology and consequently improved the method of classifying the organisms. It is a quick and more selective method for preparing large quantities of a particular gene or other DNA sequences in a test tube when the source of DNA is scanty or impure. In this technique, PCR works like a photocopying machine, for that reason, it can make billions of copies of the target segment of DNA in a few hours greatly faster than the days it will take to get the same number of copies by screening a DNA library for a clone with the desired gene and letting it replicate within host cells (Campbell et al., 2008).

The PCR is driven by controlled changes in temperature that accomplish the following three-step cycle: denaturation, annealing and extension. During each cycle, the reaction mixture is heated to denature (heat briefly to separate DNA strands); the DNA strands are then cooled to allow annealing (to allow primers to form hydrogen bonds with ends of target sequence), of short, single stranded DNA primers which are complementary to sequences on opposite strands at each end of the target sequence; finally, a heat-stable DNA polymerase extends the primers (DNA polymerase adds nucleotides to the 3′ end of each primer) in the 5′ → 3′ direction. To avoid denaturing of the enzyme of DNA polymerase during the first heating step, a special DNA polymerase enzyme is used. This enzyme is isolated from cells of a

bacteria *Thermophilus aquaticus* which lives in hot springs that withstands the heat at the start of each cycle (Bonacum et al., 2002; Campbell et al., 2008).

Only minute amounts of DNA need to be present in the starting material. This DNA can be in a partially degraded state as long as a few molecules contain the complete target sequence. The key to this high specificity is the primer. A primer is a short stretch of RNA with a free 3′ end bound by complementary base pairing to the template strand which is elongated with DNA nucleotides during DNA replication. Primer must at least consist of 15 nucleotides for high specificity. In the process, merging the opposite primer must be avoided (Maareg, 1999; Campbell et al., 2008).

Knowing the sequence of a gene allows researchers to compare it directly with the genes in other species. In this way, sequence comparisons provide clues to clear the view of the relationships among species. In the past, the relationships were dependent on morphological character data from extant taxa and fossil record. This old method leads to many different scenarios about the relationships between taxa because of the inconsiderable data provided from the fossils and extinct organisms. Today, after the advent of molecular sequencing, the huge number of new data sets has made the scenario of life more clear than before. Phylogenetic tree is used to represent the historical relationships of taxa (Campbell et al., 2008).

The simple structure of the phylogenetic tree is nodes and branches. A branch is a line that connects two nodes. Nodes symbolize the split of a lineage in evolutionary time through speciation; nodes can be either external nodes which represent the taxa or OTUs (operational taxonomic units), or internal nodes which are points representing a common ancestor of two or more other nodes (Hall 2004).

One of the major methods for genetic separation of populations is habitat fragmentation. The climatic changes and their consequences in addition to the natural catastrophes could cause the natural fragmentation of habitats. Currently, the human effects increase habitat fragmentation (Klütsch 2006). The information of habitat fragmentation and population differentiation in Yemen is few, as well as distributional ranges of faunal elements. More detailed studies for this geographical area are still need it.

Study Area

Many people think of Arabian Peninsula as a part of Asia, since it is separated from Africa by the Red Sea and joined in the north and north-east to the Asian continent as is shown on the map. From the viewpoint of geological history, this is erroneous; because the geological history of this area leads to the conclusion that the Arabian Peninsula was a part of Africa (Thompson 2000).

Geologists have observed good agreement between the geological structure of the eastern shore of the Red Sea and lands on the western side of the Red Sea which support this theory (Jokela, 1965, BIOT 2010).

Yemen is a part of the Arabian Peninsula that has an interesting geological history. The origin of Arabia was more than 500 million years ago as an integral part of northeast Africa: the Arabian and Nubian Shield were formed as one unit (Arabian-Nubian Shield) by the same forces at the same time and still as one unit less than 50 million years ago. The separation of the Arabian landmass from the African plate started about 60 million years ago along the line of the Red Sea and the Gulf of Aden by tectonic drift effects. The Arabian plate began to shift north-eastwards and impacted with the Eurasian plate about 15 million years ago. The result of this collision formed the Zagrose mountains in Iran and the mountain systems in Eurasia. Furthermore, a chain of lakes later afterwards formed the Red Sea, and several connections remined between the African plate and the Arabian peninsula along the escarpment. The rifting process continued in this region throughout that period (fig. 1). However, the African and Arabian plates were still connected through a land bridge near Djebouti. After that, the Isthmus of Suze arose, cutting the Red Sea off

from the Mediterranean. The Aden Gulf and Bab Al-Mandab straits began to sink, which allowed to form a continuous waterway between the Red Sea and the Indian Ocean. At the same time, there was a rise of the escarpment and of adjacent landmasses than sea level, which presented the mountains of Yemen and the Asir heights to their present states (Thompson 2000, Klütsch 2006).

These geological conditions imply that a biological colonization of Arabia was complicated during the drift processes. Arabian populations became separated from the African continent and within the Arabian Peninsula (Klütsch 2006). Therefore, Yemen has a highly specialized fauna and flora of peculiar interest to the taxonomic researchers and to evolutionary biologists.

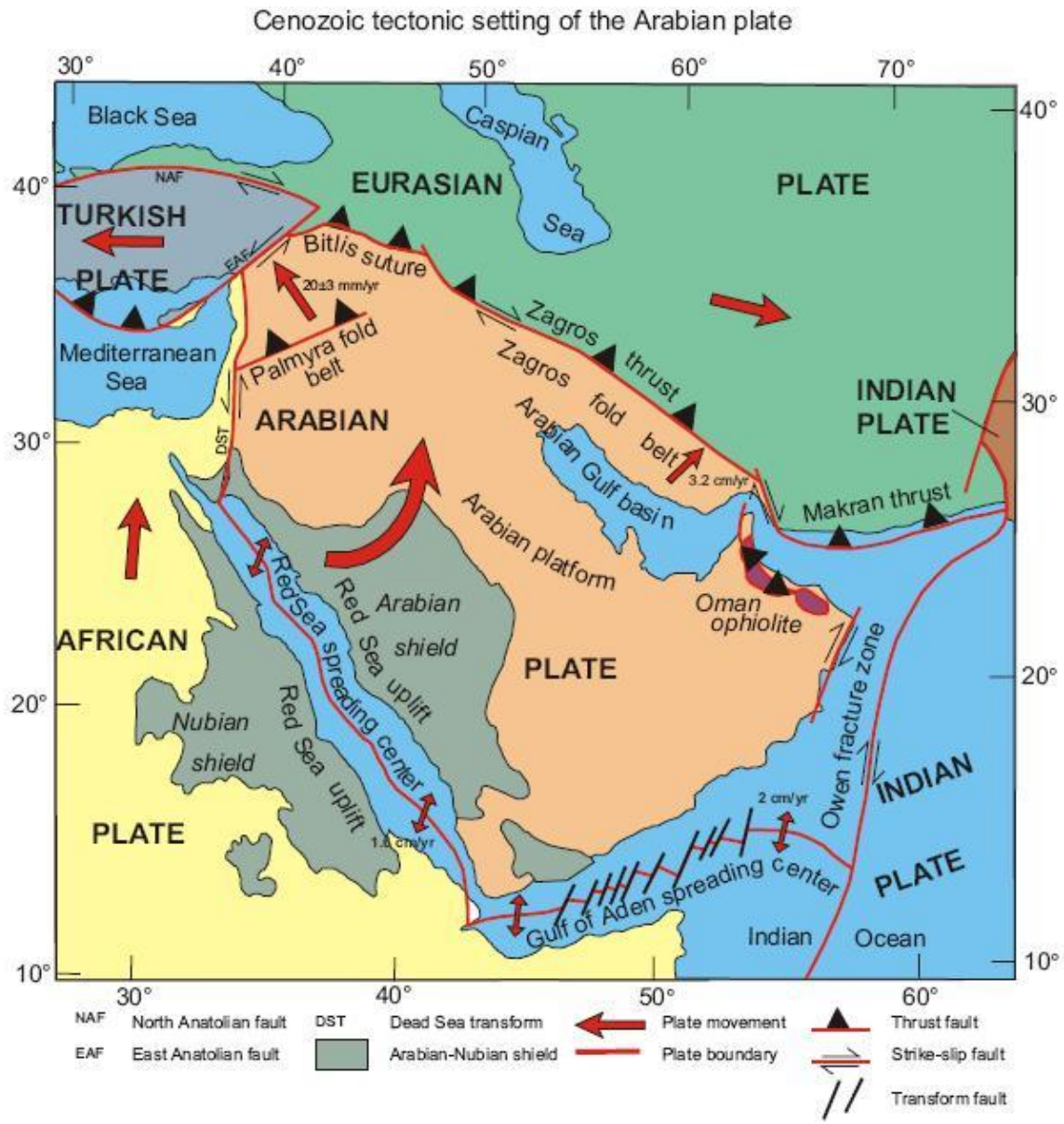


Figure 1: The Arabian plate movement (after Vincent 2008).

The reptile fauna of Yemen consists mainly of Afrotropical elements as a result of the geological history and the barrier effects of the mountains, which shows a high degree of correspondence to Somalia along with a few Palaearctic faunal elements, and occupies a unique position within the Arabian Peninsula, and a number of species seem to be restricted to this region, therefore, the reptile fauna of the peninsula is mainly Saharo-Sindian (Joger 1987). Thus Yemen belongs to the Ethiopian (Afrotropical) zoogeographical region and to the East African subregion which includes tropical Africa and tropical Arabia (Wallace 1876, Smith 1983). Other authors deviate from this classification. Kreft & Jetz (2010) unite all Arabian and Iranian mammal faunas in an 'Arabo-Sindic group' within an African region.

The Republic of Yemen occupies an area of 527,970 km² in the south and southwestern corner of the Arabian Peninsula between latitude 12° 40′ and 19° 00′ North, and 42° 30′ to 53° 05′ East longitude. It is bordered by the Kingdom of Saudi Arabia in the north, Oman in the east, the Gulf of Aden and the Arabian Sea, an extension to the Indian Ocean, in the south and the Red Sea in the west. The coastline extends for approximately 2500 km long. The northern and eastern borders face the desert of the Rub'a Al-Khali (CBD 2009).

Parallel to the western coast of Yemen are the Sarawat mountains, in which the highest mountain in Arabia, Jabal An-Nebi Shu'aib is located, attaining a height of 3666 m. These mountains are under the influence of the south-westerly monsoons (Kaul and Thalan 1979, Wood 1997). The country lies within the northern stretches of the tropical climatic zone and its border with the sub-tropical climatic zone. The extreme differences in elevation are largely responsible for the great variations in temperature and climate over the country. The annual rainfall varies widely, from 50

mm along the coastline, rising with the topography to more than 1200 in the western highlands and dropping again to below than 50 mm in the desert interior, (Al-Jumaily 1998, Busais 2003, CBD 2009). Temperature depends on elevation, and in the coastal areas is determined by distance from the sea. Mean annual temperatures range from less than 12°C in the highlands with occasional freezing to 35°C in the coastal plains.

Regarding to (Busais 2003, NIC 2003) the climate of Yemen is divided into five major land systems according to the topographic divisions. Thus, the topographic divisions of Yemen consist of five topographic regions besides the islands (fig. 2), as the following:

The Coastal plain:

Coastal plain extends discontinuously along the Yemeni coasts and includes two different coastal regions: Southern and southern east plain in the Gulf of Aden and the Arabian Sea, and the Western plain in the Red Sea at the west, which is dissected by several plateaus and mountains reaching directly to the seawater. It is covering approximately 11% of the area of Yemen.

The coastal plain consists of mostly sand and gravel plains; it includes:

Tehama plain,

Tuban-Abyan plain,

Maifa'a-Ahwar plain,

The Eastern coastal plain of Al-Mahra governorate.

It is characterized by a humid and hot climate all over the year, the mean temperatures are usually over 30° C. Rainfall is irregular, approximately 50 – 100 mm / year, it falls during the winter season and sometimes during the tempests in July and August throughout the monsoon. Usually in the morning, heavy dew occurs. The Coastal Plain is of agricultural importance, especially the Western coast plain, Tehama plain, due to a lot of wide valleys running across it and characterized by accumulation of high running water. The Southern Coastal plain hardly ever attains more than 20 to 30 km in width and is best developed in the hinterland of Aden along the lower course of Wadi Tuban, extending as far inland as 60 km. In some locations, the coastal plain is narrow, between the entrance of Wadi Hajr and Al-Mukalla, around Ras Fartak and elsewhere in other locations mountains reach the coastline (Schaetti and Desvoignes 1999).

High Mountains:

This sector extends from the far north to the far south, which was in the past subject to tectonic movements through geologic time that lead to form series of faults parallel to the Red Sea and Gulf of Aden. It is a volcanic region with an elevation between 1000 and 3600 m. It comprises of the highest mountains in the Arabian Peninsula with an average height of 2000 m., the largest height in Jabal An-Nebi Shu'aib at 3666 m. The rainfall is between 500 to more than 1000 mm in the western mountainous highland region occurring in two periods: the first, from March to May and the second, from July to September. These high mountains include valleys bounded by step sided mountains directly facing the Tehama plain. The most important valleys of these mountains are: Wadi Moor, Wadi Haradh, Wadi Zabied,

Wadi Seham and Wadi Rasyan which reach the Red Sea. Other Wadis Tuban, Bana and Hadhramout extend to the Gulf of Aden and the Arabian Sea.

Mountain Basins:

These basins comprise the valley plains which are situated between the mountains. Most of them lay in the eastern part of the High Mountains. They are represented by the plains of Sana'a, Yarim, Ma'abar, Al-Abr, Amran and Sa'ada Basins. The climate is in the range of 25°C with lower rainfall and humidity compared to the High Mountains.

Yemen Plateaus:

The Yemeni plateaus are located in the eastern and northeastern parts of the high mountains and parallel to them. They widen towards Rub 'a Al-Khali desert, and decrease in elevation. The surface of the plateau slopes gradually towards the north and the east. The surface of these plateaus compose of rocky deserts crossed by several huge valleys like Wadi Hadhramout and Harieb.

The second largest desert valley in the Arabian Peninsula is Wadi Hadhramout with an area of over 20,000 km^2 (Villwock 1991). This valley starts at an approximately 70 km wide valley at the eastern end of Ramlat As-Sabatain. The edges of the Plateaus are deeply cut at a 90 degree angle producing walls of more than 300 m height. The water is constant in Wadi Duan, Wadi Adim and below Tarim (Bent 1894, Scortecci 1963) where Wadi Hadhramout turns southeastward and becomes Wadi Al-Masilah which enters the Gulf of Aden near Sayhut (Schaetti and Desvoignes1999).

The Desert:

The desert comprises of a sandy sector without any plant cover except the locations where rainwater flows. It has an elevation of 500 – 1000 m elevation above the sea level. It decreases in elevation towards the center of Rub'a Al- Khali in the northeast.

The climate is characterized by a high temperature with a large temperature range, very rare rainfall, and low humidity.

The Islands of Yemen

There are 112 island territories distributed in Yemeni seawater located in the Red Sea, Gulf of Aden and Arabian Sea. The largest Islands in the Red Sea are Kamaran (181 km^2) in addition to Huneish Archipelago and Mayoun Island which lies in the narrow strait of Bab Al-Mandab at the southern part of the Red Sea. On the other hand, the most important and biggest Islands in the Arabian Sea are the Socotra Archipelago. The Socotra Archipelago is situated in the north-western part of the Indian Ocean and comprises the four islands: Socotra (3600 km^2), Abdel Kuri (162 km^2), Samha (45 km^2) and Darsa (10 km^2). The climate is monsoonal. The Islands are separated by relatively shallow seas and from the mainland by a deep trench (Wranik 1998, NIC 2003, CBD 2009).

Socotra Island is composed of a basement complex of igneous and metamorphic rocks of Pre-Cambrian age, overlain by sedimentary rocks, mainly limestone and sandstone.

The topography of Socotra Island is divided into three main zones (fig. 3) Wranik (1998, 2003) as the following:

1. The coastal plains,
2. The limestone plateau,
3. The Haghir Mountains.

The Socotra Archipelago is distinguished by a unique geology and a rich variety of plant and animal species including an exceptional number of endemic species as a result of long isolation. This unique position, has made the archipelago a ‘living laboratory’ of remarkable biogeographic and evolutionary interest for wildlife conservation.

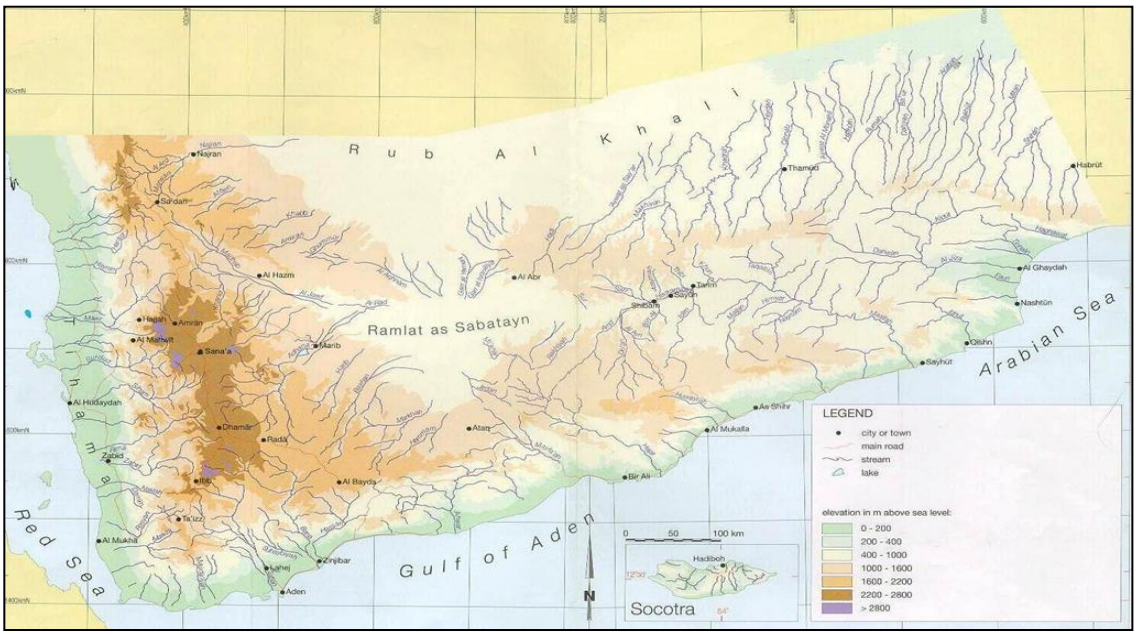


Figure 2: Topographic map of Yemen (after the National Information Center NIC).

Figure 3: Topographic map of Socotra Archipelago (after http://mapsof.net/socotra/static-maps/).

Biodiversity Status

Yemen contains a variety of habitats which range from coastal mangroves, shrub lands and dunes along the coastal plains to the eastern deserts and an array of mountain habitats that reach elevations around 3666 m at the tip of the mountain of Jabal Al-Nabi Shauib, the highest point in the Arabian Peninsula (Wood 1997, CBD 2009).

Yemen has a special geographical position between the Arabian Peninsula and Africa. This location is considered the junction point of the Red sea and Arabian Sea and has given Yemen different climatic features, and the variety of the topographic of the country. These factors are favorable for the existence of divers ecosystems along with a high level of biodiversity (CBD 2009).The natural process of desertification led to an isolation of these areas into fragmented habitats (Thompson 2000). Nowadays habitat fragmentation and desertification is rapidly progressing due to anthropogenic influences (Klütsch 2006).

Status of the Flora of Yemen

Yemen has a rich flora and heterogeneous. Species diversity is a result of various topographical features and considerable climatic changes in former periods. These features enabled different species to survive in the different ecological habitats. There are more than 3730 plant species recorded in Yemen belongs to more than 1006 genera related to 175 families and several studies indicated that there are more than one hundred and fifty species are endemic in the mainland. The family Poaceae is the largest family in Yemen. It is represented by 322 species, proceed it by family Astraceae with 223 species (Al-Khulaidi 2000, Al-Dubaie 2004, Aqlan 2008, CBD 2009). The flora of Socotra Archipelago is unique; it has a high level of endemism similar to other oceanic islands. Socotra Archipelago contains of approximately 825 plant species, 307 (about 37%) of which are endemic related to 15 endemic genera (Miller and Miranda 2004).

The majority of endemic taxa in Yemen are associated with mountainous areas, which provide a rich variety of ecological niches and offer a degree of environmental stability during periods of climatic changes. Endemic species are numerous and found in genera of both tropical and temperate origin, though large proportions are in succulent genera such as *Aloe sp*., *Caralluma sp*. and *Euphorbia sp*. The families: Asteraceae, Apocynaceae, Euphorbiaceae, Acanthaceae and Boraginaceae, respectively, are the largest family in Yemen. These families contain approximately one hundred and seventy two endemic species (Wood 1997, Aqlan 2008, CBD 2009).

Status of the Terrestrial Fauna of Yemen

Yemen has a rich and diverse fauna since of the wide range of habitats in the country and due to its position at the juncture of two major biogeographic regions, Afrotropical and Palaearctic (Euro-Asiatic) regions (Wheatley 1997).

Mammals

There are seventy two recorded species of land mammals in Yemen representing eight orders including bats (Chiroptera). The largest group of mammals belongs to the order of Chiroptera with 24 species (Al-Jumaily 1998).

Several mammals are relatively large species which are rare in other parts of Arabia such as the Arabian Mountain Gazelle (*Gazella gazella*), Ibex (*Capra ibex nubiana*), Baboon (*Papio hamadryas*), Arabian Red Fox (*Vulpes vulpes arabicus*), Sand Fox (*Vulpes ruppelli*), Blanford's Fox (*Vulpes cana*), Striped Hyena (*Hyaena hyaena*), Arabian Wolf (*Canis lupus arabs*), Jackal (*Canis aureus*), Arabian Leopard (*Panthera pardus nimr*), and possibly the Cheetah (*Acinonyx jubatus*). It is notable that seven mammal species are now considered endangered including three of the four species of gazelle, and another three species of the Cheetah, Arabian Oryx and the fourth gazelle, the Queen of Sheba’s Gazelle are now extinct in the wild. Furthermore, most large mammals have long since been hunted into extinction in Yemen where firearms abound and a large proportion of the natural forests have been cut down. With some dedication and luck, ecotourists may still spot rare land animals such as the Arabian leopard, hyena, Hamadryas baboon, honey badger, hedgehog, ibex and fox. For long time, large mammals have been under considerable pressure and some of which vanished from the country and most of the others became rare

and threatened. Over the last century, four species have been killed and became extinct. The Nubian ibex (*Capra nubiana*), the Arabian leopard (*Panthera pardus nimr*), Arabian oryx (*Oryx leucoryx*) are and the three Arabian gazelles listed above are decreasing sharply and have become rare as a results of continues hunting and absence of protection, breeding and re-introduction programmes (Obady 1993, CBD 2009). The widespread use of weapons among the local public has endangered these species and many other species. For this reason, the local authority has called to preserve several areas as a natural protected area.

Reptiles and Amphibians

Around 119 species of reptiles and amphibians have been recorded in Yemen. The amphibians include around seven species belonging to three families: Bufonidae, Hylidae and Ranidae. The reptiles include 74 species of lizards, 28 snakes and 3 amphisbaenia, all belonging to the order Squamata which comprises the largest reptilian group. Turtles (order Testudinata) are represented in Yemen by six species, one terrestrial species (*Geochelon sulcata*), one freshwater species (*Pelomadosa subrufa*) and four species of marine turtles. The latter were recorded from the Yemeni waters. These species are:

1- *Chelonia mydas* (Green turtle)

2- *Eretmochelys imbricata* (Hawksbill turtle)

3- *Caretta caretta* (Loggerhead turtle)

4- *Dermochelys coriacea* (Leatherbacks turtle)

Caretta caretta was recorded only from Socotra Archipelago (Al-Safadi 1991, Obady 1996, CBD 2009).

There are 28 snake species related to seven families in Yemen, including Typhlopidae, Leptotyphlopidae, Boidae, Colubridae, Atractaspididae, Elapidae and Viperidae (Gasperetti 1988, Schätti and Gasperetti 1994, Obady 1996, Busais 2003, Busais and Al-Jumaily 2005).

Seventy four species of lizards recorded in Yemen belong to 25 genera. These species related to the families of Agamidae, Chamaeleonidae, Gekkonidae, Lacertidae, Scincidae, Varanidae and Trogonophidae (Amphisbaenians). The biggest

family lizard in Arabian Peninsula and Yemen is the family of Gekkonidae. This family represented in the mainland of Yemen by the following genera: *Bunopus, Cyrtopodion, Hemidactylus, Pristurus, Ptyodactylus, Stenodactylus* and *Tropiocolotes* (Arnold 1986, Schätti and Gasperetti 1994, Obady 1996, Schätti and Desvoignes 1999). Furthermore, in the Socotra Archipelago it is represented by: *Haemodracon, Hemidactylus, Pristurus* (Joger 2000; Wranik 2003; Rösler and Wranik 2004, 2006).

In the Socotra Archipelago, approximately 34 species have been reported, and 27 of them are endemic, with about 40% endemic genera, including the genera of *Haemodracon*, *Hakaria*, *Pachycalamus*, *Ditypophis* and *Hemerophis* (Joger 2000, Rösler and Wranik 2004).

The geckoes have far more representatives in Arabia than any other reptile family. They appear to constitute approximately 40 % of the lizards' species and nearly 30% of all terrestrial reptiles in the area (Arnold 1977).

The exclusive geographical position of Yemen between Asia, Arabia and Africa, and the junction point of the Red Sea and the Indian Ocean has given Yemen different climatic and topographical features which are favorable for the existence of diverse ecosystems along with a high level of biodiversity. Therefore, the country has a rich and diverse fauna and flora (Obady 1996, CBD 2009).

Family Gekkonidae:

1825 Geckotidae Gray, Ann. Philos. (2), 10, p. 198.

1871 Gecconidae Cope, Proc. Amer. Assoc. Adv. Sci., 19, p. 236

1883 Eublepharidae Boulenger, ann. Mag. Nat. Hist. (5), 12, p. 308

1884 Geckonidae Boulenger, ann. Mag. Nat. Hist. (5), 14, p. 119

Gekkota, originally erected for lizards commonly known as geckoes (geckos or gekkos) now usually placed in a single family Gekkonidae (Donnellan et al., 1999). The spelling (Gekkonidae) is based on that of the genus *Gekko* (Laurenti 1768). This family contains the most common reptiles of the order Squamata. It includes so far the greatest number of living genera and species and it represents more than 25% of genera and species of lizards (Kluge 1987). One genus of this family is *Hemidactylus* which alone accounts for nearly 10% of the total (Kluge 1969). This family has been of great scientific interests for centuries.

The general form of members of this family is more or less depressed; no symmetrical shields cover the head; eyes with vertical or round pupil moving freely beneath a transparent membrane that is present in most species; eyelids vestigial or more or less well-developed and connived; tympanum more or less distinct; dentition pleurodont, teeth numerous, small, hollow at base, feebly nicked anteriorly, protrusable but non-extensile; skin usually soft, that of the dorsum generally bearing granules or tubercles, more rarely imbricate, cycloid or hexagonal scales like those on the ventral surface; limbs well developed, pentadactyle or inner digit vestigal; digits too variable, clawed or clawless, the claws sometimes retractile; tail variable,

cylindrical or depressed, or compressed and crested (as in certain male *Pristurus*), slender and tapering or thick and sometimes carrot-shaped, usually fragile, rarely prehensile (Loveridge 1947).

Geckoes are distributed worldwide. The greatest diversity of species inhabits the deserts, tropical and sub-tropical regions. Many geckoes differ from other lizards in having a voice used for communication in the dark. In East African geckoes (as well in Yemen) this is mostly just a squeak, likely to be heard when the animal is seized, however, some Asian geckoes have a strident grunt, and a number of Southern African species have a repertory of clicks and barks (Spawls 2002).

The genera of *Hemidactylus* and *Pristurus* contain the most number of Yemeni species in the family of Gekkonidae with 22 species (Obady 1996). However, the investigation on the Yemeni lizards in the mainland has not been wide and many records need verification.

Genus *Hemidactylus* Oken, 1817

Hemidactylus is a genus of the family Gekkonidae, suborder Lacertilia (Sauria), order Squamata, Class Reptilia. This genus is one of the most species-rich genera of the family Gekkonidae which contains some 79 species (Kluge 2001), however this number has increased slowly to more than 83 recognized species (Bauer and Pauwels 2002; Baha el Din 2003, 2005; Henle and Böhme 2003; Carranza and Arnold 2006; Bauer et al., 2006b). After the discovery of the most recent species from Kenya, Myanmar, Cape Verde Islands, India, Pakistan and Socotra Island the number of species should reach to 93 species (Sindaco et al., 2007, 2009; Zug and McMahan 2007; Arnold et al., 2008; Giri 2008; Giri and Bauer 2008; Bauer et al., 2008; Giri et al., 2009; Ullenbruch et al. 2010, Agarwal et al. 2011).

Most of *Hemidactylus* species are listed together with their synonymies by Loveridge (1947), Wermuth (1965) and Kluge (1991) (Carranza and Arnold 2006).

The origin of this genus is Africa (Kluge 1969). More precisely, its main centre of speciation is in East Africa: Somalia, Kenya, Ethiopia, and Eritrea which host more than 40 species of *Hemidactylus*, most of them are endemic (Parker 1942, Lanza 1983, Spawls 2002, Brogard 2005, Largen and Spawls 2006, Sindaco et al., 2007). It is mainly nocturnal and often climbs. These geckos occur naturally through much of tropical Asia and Africa and in the intervening more arid areas of Northeast Africa and Southwest Asia. Furthermore, they have extended into the Mediterranean region and reached South America apparently by natural transmarine colonization (Kluge 1969, Carranza and Arnold 2006, Bauer et al., 2006b). Obviously, this genus is able

to perform long distance natural and anthropogenic distribution, followed by colonization of new areas (Kluge 1969, Carranza et al., 2000; Vences et al., 2004).

Most of the species of *Hemidactylus* exhibit relatively small geographical ranges being confined to southern Asia and Africa, and just eight species are responsible for most of the huge geographical area covered by the genus, these species are: *H. mabouia, H. turcicus, H. brookii, H. frenatus, H. garnotii, H. persicus, H. flaviviridis* and *H. bowringii.* The first five in particular are widespread and present in both the Old and New Worlds, with *H. mabouia* also occurring on islands in the Atlantic, and *H. frenatus* and *H. garnotii* being widespread in the Pacific. For this reason sometimes these forms are called 'weedy' species (Kluge 1969, Carranza and Arnold 2006).

Hemidactylus is characterized by digits with dilated pads at their base, lamellae on ventral side of pads divided longitudinally; distal phalanges free. All digits clawed. Pupil vertical. Usually 2-3 postmental shields, the first pair in contact behind the mental. Males with pre-anal or femoral pores. Dorsum with granular, subimbricate, uniform scales or more often with enlarged tubercles (Baha el Din 2006).

The traditional morphological characters used to classify this genus are: body length, number of dorsal tubercles, number and position of scansors (lamellae), number of preanal-femoral pores in males, and the number of the tail rings (Kluge 1969, Loveridge 1947, Fritz and Schütte 1987, Spawls 2002). Other characters were added from (Bauer and Pauwels 2002; Giri et al., 2003). Coloration patterns are not used to differentiate among species since these geckoes have the ability to change skin color.

There are complications in classifying the genus of *Hemidactylus* as the species are extremely similar to each other, for example in the number of scansors and tubercles, the size of dorsal scales and absence or enlarged dorsal tubercles, when present, their number, size and shape and other morphological characters. The degree of overlap for approximately every characteristic makes it often difficult to exactly identify a species. These factors have in many cases led to overlooking of fairly obvious and consistent morphological and ecological differences amongst various populations (Kluge 1969, Spawls 2002, Carranza and Arnold 2006).

In the study of *Hemidactylus* geckos using mitochondrial DNA sequences by Carranza and Arnold (2006), five major clades are discernable that have well-supported value, they are:

1) Tropical Asian clade,
2) African *Hemidactylus angulatus* clade,
3) Arid clade,
4) *Hemidactylus mabouia* clade,
5) African-Atlantic clade.

According to the previous study, the positions of the Yemeni *Hemidactylus* species fall within two of these clades: the large group is within the ‘arid clade’ and only one species (*H. flaviviridis*) is within the ‘tropical Asian clade’.

The current study focuses on the species inside the arid clades from the mainland of Yemen.

Previous Studies on *Hemidactylus* in Yemen

Yemen has not received sufficient scientific research studies due to rigorous political rule which limited scientific expeditions targeting this country. Previous work concentrated mainly on recording which species occurred in the country. Research on the lizards in the mainland has not been extensive and some records need confirmation and a critical revision. The chronological summary of some of the early works is controversial. The research reports presented in the 1770's and the beginnings of the twentieth century were based on information gleaned from traveling naturalists rather than actual surveys. Yemen was overlooked for two centuries by scientific researchers due to instability of the political rule beginning with the civil wars and ending with British attempts to dominate international sea channels. Furthermore, diseases were widespread in the area and took hold of the lives of the earlier researchers that came to discover this area such as Forskål and his colleagues. These factors cause insufficient exploration in Yemen.

The first European scientific expedition investigating of the reptiles of Yemen started with the Royal Danish expedition of 1762-1763. Forskål was selected as the biologist for this mission. He collected scientific specimens in Egypt and along the Arabian shores of the Red Sea on the way to Yemen (Forskål 1775). Forskål died in Yemen by Malaria in 1763, then C. Niebuhr published his notes posthumously, but there were no *Hemidactylus* specimens among his collection.

Research studies reported afterwards were as follows:

Parenti and Picaglia (1886), and Boettger (1892) reported *Hemidactylus flaviviridis* from Aden.

Anderson (1895, 1901) published the results of the material collected by Colonel Yerbury from Aden and its surrounding area; he mentioned that the Yerbury's collection comprised 11 species of reptiles to the fauna of Aden and Lahj, which include *Hemidactylus flaviviridis, H. sinaitus* and *H. yerburii*.

Steindachner (1903) published a report of the herpetological material including a collection of *H. turcicus* and *H. yerburii* taken by W. Hein from the Mahrah littoral between Qishn and Ras Fartak.

Schmidt (1953) collected three species from northern part of Yemen, four specimens from Sana'a related to *H. t. turcicus*, two specimens from Taiz related to *H. flaviviridis* and thirteen specimens from Taiz and two from Al-Hudaidah related to *H. yerburii.*

Haas and Battersby (1959) studied amphibians and reptiles from Popov's collection during 1950 to 1953. The authors described *Hemidactylus shugraensis* from Shugra, Abian coast. This collection also contained *H. yerburii* from a drainage complex in Seiyon, Hadhramout.

Arnold (1977) recorded *Hemidactylus sinaitus* and *H. yerburii* from the Aden area, Mahfid and Al-Mahra, *H.* cf. *homoeolepis* from the Arabian Peninsula including a specimen from Shugra, and *H. turcicus parkeri* from Hadhramout.

Al-Badry and Al-Safadi (1982) recorded *H. t. turcicus* from Sana'a, *H. turcicus parkeri* from Al-Hudaidah, *H. yerburii* from Sana'a-Al-Hudaidah road and *H. flaviviridis* from Taiz and also from Al-Hudaidah.

Arnold (1986) presented a key and checklist for the lizards and amphisbaenians of Arabia, and he included five Yemeni species of *Hemidactylus* namely: *H. flaviviridis* from the coastal area, *H. homoeolepis* from South of Yemen (Shugra and Socotra Island), *H. sinaitus* from Aden and Shugra, *H. turcicus* and *H. yerburii*. The latter two species were also from south of Yemen.

Fritz and Schütte (1987) observed and collected three species from the north of Yemen: *Hemidactylus yerburii*, *H. turcicus parkeri* and *H. flaviviridis*.

Schätti and Gasperetti (1994) discussed the status of amphibians and reptiles of Southwest Arabia including *Hemidactylus flaviviridis, H. sinaitus, H. turcicus* and *H. yerburii yerburii* from Yemen; they suggest that the occurrence of *H. sinaitus* in Aden area is probably due to accidental introduction, and at *H. turcicus* is in need of much further investigations in order to clarify their distribution and classification to differentiate with related taxa.

Obady (1996) published a popular account of the herpetofauna of Yemen and mentioned three species of *Hemidactylus* collected from several locations from the mainland, these are: *Hemidactylus flaviviridis, H. turcicus* and *H. yerburii*.

Schätti and Desvoignes (1999) studied the herpetofauna of Southern Yemen, which included six species of *Hemidactylus*, these were: *H. flaviviridis, H. homoeolepis*, *H. lemurinus, H. sinaitus, H. turcicus* and *H. yerburii*.

The report of endangered animals in Yemen (2006) included the *Hemidactylus persicus, H. flaviviridis, H. homoeolepis, H. lemurinus, H. sinaitus, H. turcicus* and *H. yerburii* to the list of endangered lizards in Yemen.

From the above, it is obvious that the information on the classification and distribution of *Hemidactylus* in this geographical area is not fully known, neither studied systematically. Furthermore, eight species of this widespread genus are known for the Socotra Archipelago (Obady 1996; Rösler and Wranik 1999, 2006; Joger 2000; Wranik 2003; Sindaco et al., 2009). However, the number of species is not clear for the mainland of Yemen.

Therefore, there is still need for systematics and phylogeny studies on the classification and distribution of this genus in Yemen as well as other lizards. For that reason, this study is important to clear the taxonomic status of the genus *Hemidactylus* in the mainland of Yemen by using both of the morphological and molecular characters.

The Aims

This study seeks to identify specimens of *Hemidactylus* geckoes from several localities in Yemen using morphological and molecular approaches. Therefore, the aim of the present study is to analyze the genetic composition of the Yemeni populations of *Hemidactylus* and compare them morphologically.

The main objective in this research is to construct a phylogenetic tree of the genus *Hemidactylus* residing in the mainland of Yemen.

Focus was put on the following central questions:

1) How many *Hemidactylus* species actually occur in the mainland of Yemen, and how are they distributed over the country?

2) Is there a relationship between species from the mainland and the Socotra Archipelago?

3) Are the species *Hemidactylus homoeolepis* and *H. turcicus* found on the mainland?

4) Is the species *H. lemurinus* found in Yemen?

Materials and Methods

The Systematic methods used to classify the living organisms depend on the morphological characters and recently on the molecular characters. This study depends on both the morphological and molecular characters to identify the Yemeni species of the genus *Hemidactylus*.

The phylogenetic analysis clearly distinguishes eight clades of Yemeni *Hemidactylus* taxa that found on the mainland. Depending on the phylogenetic results, specimens for each clade was studied alone as a separate group.

The Samples

One hundred and eighty five samples of geckoes were collected from August 2007 to February 2008 from thirty two localities in Yemen (table 1, fig. 4). Moreover, additional samples were added during February 2009. In addition, two samples related to *Hemidactylus angulatus* from Niger were deposited in the State of Natural History Museum, Braunschweig, Germany (NHM-BS) 'Naturhistorischesn Museum Braunschweig'.

Furthermore, the Yemeni samples were compared with 26 known samples from the Museum of Zoology (MTKD) 'Museum für Tierkunde Dresden' as well as 19 samples from the Museum of the Alexander Koenig for Zoological Research, Bonn (ZFMK) 'Zoologisches Forschungsmuseum Alexander Koenig; the specimens, which were compared from the MTKD and ZFMK museums were from Yemen as well as other Arabian and African countries. In addition, tissues referring to the

known *Hemidactylus* species collected from Socotra and identified by Prof. Dr. Ulrich Joger were extracted, sequenced and examined within this study.

The samples were collected by hand and injected with Ethanol. After that, they preserved in plastic containers containing 95% - 99% Ethanol. The best method to collect geckoes when they are between rocks or fixed to high roofs is by tossing Ethanol in a syringe on to the target that will help the target to drop easily.

The collected samples are deposited in the Natural History Museum in Braunschweig (NHM-BS), with a list of samples with reference numbers of tissues as well as detailed information on their locality and the date of collection is given in appendix I.

Table 1: : The coordinates and altitude for each locality of study area.

No.	*Locality*	*Altitude*	*North*	*East*
1	Ad-Daliel, Ibb	1608 m	14^{o} 06`	44^{o} 11`
2	Aden	4 m	12^{o} 50`	45^{o} 02`
3	Al-Habielain, Adh-Dhale'a	1470 m	13^{o} 41`	44^{o} 44`
4	Al-Harshiiat, Al-Mukalla	74 m	14^{o} 34`	49^{o} 09`
5	Al-Mahfad, Abian	668 m	14^{o} 03`	46^{o} 54`
6	Al-Makhader, Ibb	1726 m	14^{o} 06`	44^{o} 10`
7	Al-Mnyasa, Lowder, Abian	986 m	13^{o} 52`	45^{o} 55`
8	Al-Udain, Ibb	1957 m	13^{o} 58`	44^{o} 08`
9	Amran	2239 m	15^{o} 39`	43^{o} 56`
10	Aryab, Abian	2216 m	13^{o} 56`	45^{o} 42`
11	Ash-Shihr, Hadramout	12 m	14^{o} 45`	49^{o} 36`
12	As-Suhool, Ibb	1690 m	14^{o} 00`	44^{o} 10`
13	At-Taweela, Al-Mahweet	2197 m	15^{o} 28`	43^{o} 32`
14	Ba'dan, Ibb	2146 m	13^{o} 58`	44^{o} 11
15	Ghail Bawzeer, Hadhramout	111 m	14^{o} 47`	49^{o} 22`
16	Hadda, Sana'a city, Sana'a	2306 m	15^{o} 17`	44^{o} 11`
17	Ibb Univ., Ibb	1960 m	13^{o} 58`	44^{o} 09`

Continuation of table 1.

No.	*Locality*	*Altitude*	*North*	*East*
18	Jebla, Ibb	2092 m	13° 55`	44° 09`
19	Lahj	79 m	13° 00`	45° 54`
20	Ma'reb	1082 m	15° 27`	45° 20`
21	Mas'abein, Shaikh Othman, Aden	12 m	12° 55`	44° 59`
22	Mebar, Thamar	2327 m	14° 47`	44° 17`
23	Mukairas, Abian	2170 m	13° 56`	45° 40`
24	Radfan, Lahj	664 m	13° 32`	44° 50`
25	Radman, Al-Baidha		14°08'	45°17'
26	Sana vill., Sana'a	2435 m	15° 17`	44° 10`
27	Sana'a	2254 m	15° 23`	44° 14`
28	Tebala, Ash-Sheher,	92 m	14° 49`	49° 35`
29	Thamar	2436 m	14° 34`	44° 23`
30	Tour Al-Baha	690 m	13° 10`	44° 11`
31	Yariem, Thamar	2625 m	14° 17`	44° 48`
32	Zindjebar, Abian	71 m	13° 14`	45° 15`

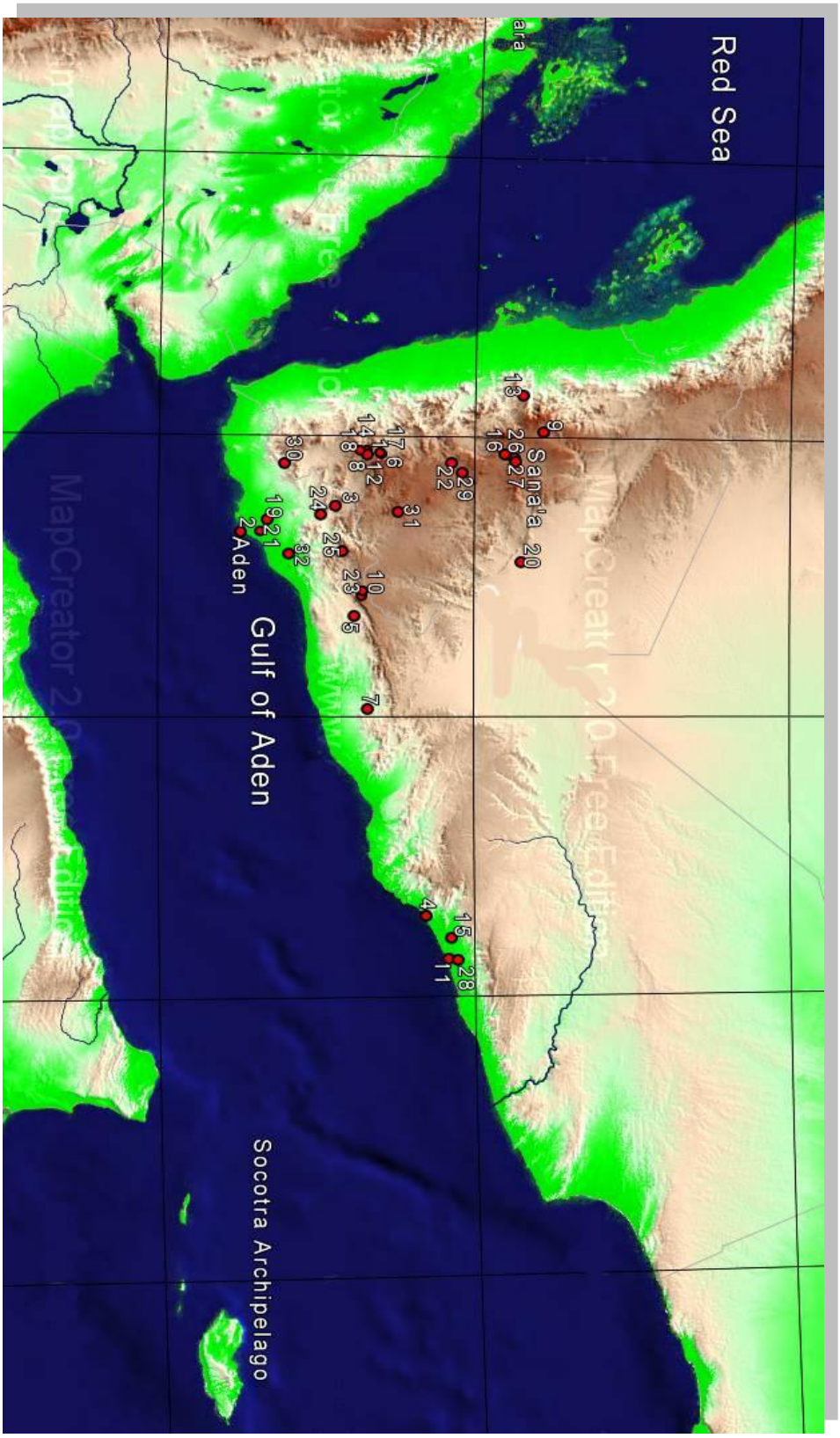


Figure 4: the localities of collected samples from the mainland of Yemen.

A- Phylogeny

The DNA of one hundred and eighty five specimens of Yemeni *Hemidactylus* specimens and two samples of *Hemidactylus angulatus* from Niger were extracted and sequenced throughout this study (fig. 6). In addition to several representative taxa collected from the Genbank related to Yemen and neighboring countries examined with the sequences of the previous samples with 12S and cytochrome *b* based on the result study of Carranza and Arnold (2006). Furthermore, twenty four sample tissues for known Socotran species were extracted and sequences with 12S gene (fig. 6).

For the nuclear gene (PDC), three sequences of known species aligned with sequences used throughout this study collected from the Genbank depending on the study of (Bauer et al., 2008) (table 8, 9).

DNA Extraction

DNA was extracted by using a standard salt extraction protocol (modified after Bruford et al., 1992), as the following:

- Adding a small piece of tissue (from heart, tail or tongue) to the 410 µl of extraction buffer plus 80 µl of 10 % SDS and 10 µl of proteinase K. Afterwards incubate 55°C for 4h, or 37°C over night.
- After the tissues were completely histolyzed, centrifuge in 5 minutes 13000 rpm, transfer supernatant in a new vessel + 180 µl NaCl. Mix it (turn Eppi. ca. 50 times or vortex it 30 seconds).
- After that, centrifuge in 5 minutes 13000 rpm centrifuge, pipette transfer supernatant quickly in a new vessel + 420 µl ice-cold Isopropanol (mixed gently).
- After transferring into a new vessel, centrifuge in 5 minutes 13000 rpm centrifuge, and carefully remove and discard supernatant.
- Add 250 µl 80 % Ethanol for washing (turn Eppi. ca. 30 times) and carefully remove and discard supernatant.
- Repeat the previous step.
- Remove the alcohol completely by dry pellet for 15 to 30 minutes in the vacuum centrifuge.
- After removing the alcohol, dilute DNA in 100 µl ddH_2O and keep it at room temperature for 1 h., then use it or freeze at -20 °C.

- To determine the approximate concentration and quality of the extracted DNA, 3 µl of each DNA solution is loaded onto a 1.0 % agarose gel containing ethidium bromide.

Agarose Gel Electrophoresis

Gel electrophoresis is used in many fields of biology and biochemistry. The results can be analyzed quantitatively by visualizing the gel with UV light and a gel imaging device. The image is recorded with a computer operated camera, and the intensity of the band or spot of interest is measured and compared against standard or markers loaded on the same gel. Depending on the type of analysis being performed, other techniques are often implemented in conjunction with the results of gel electrophoresis, providing a wide range of field-specific applications. In the present study, agarose gel electrophoresis was used to check the DNA extraction, the PCR results and the PCR results after the purification step. This was achieved by adding 3 µl of each DNA solution onto a 1.0 % agarose gel containing ethidium bromide and visualized under ultraviolet light. Gel electrophoreses were prepared by using the following protocol:

- Adding 0.5g of agarose to 50 ml TAE buffer into a 250 ml bottle which is used for gel electrophoresis. Mix by swirling then put into the microwave for about 1.5-3 minutes.
- Turn off the device and mix the solution once or twice during the microwaving. Add 1.0 µl of the Ethidium Bromide and mix.
- Seal the horizontal gel apparatus and insert a comb until its base is 1 mm from the base of the gel to make pores.
- Pour molten agarose onto a gel plate to a depth of 4 - 8 mm while avoiding bubbles. Leave in order to solidify.

- Insert the gel tray to a proper position in the electrophoresis chamber, after that fill the gel stand with buffer TBE until it covers the gel completely.
- Remove the comb, then add 2 µl of 6X Loading Dye to 3 µl of each DNA sample. Mix well then inject the mixture into the pores.
- Close the lid of the gel electrophoresis chamber and apply a current (100 V for 20 minutes).
- Remove the lid of the chamber and transfer the gel tray to the photography device to visualize the DNA bands.

Amplification of target fragments

Two mitochondrial and one nuclear genes are sequenced throughout this study (the partial cytochrome *b* gene, the partial 12S ribosome RNA gene, and the partial Phosducin gene).

Polymerase chain reaction (PCR) with specific primers situated in the flanking regions of the target fragments were performed to amplify the fragments of interest. The primers and the profiles used in this study are shown in table 2 and 3.

PCR was performed in 25 µl volume of solution containing 0.1 µl of Taq polymerase, 1 µl of primer I, 1 µl of primer II, 0.5 µl of DNTPs, 5.0 µl of buffer (containing 1.5 mM $MgCl_2$) pH 8.5, 3.0 µl of template DNA and the rest is ddH_2O until it reaches the requested volume. The PCR reaction was performed in a Biometra T-Gradient thermocycler with different temperature profiles (table 4) depending on the primers and the target fragment according to the following programs. Negative extraction controls as well as negative PCR controls (without DNA extraction) were used in each step.

The PCR was checked on 1.0% agarose gel to test the existence of the DNA amplification and to know the size of the DNA fragments. In some cases, the PCR process was repeated and the DNA product was re-amplificated under the same conditions as above but with 1.5 or 1 µl of template DNA instead of 3.0 µl (table 3).

Table 2: Primers used for amplification and sequencing of mitochondrial genes from (Kocher et al. 1989) and nuclear gene from (Bauer et al., 2007).

Gene	*Primer*	*Sequence*
Cyt *b*	SMT-A (L-14995)	5'-CAACATCTCAGCATGATGAAACTTCG-3'
	SMT-F (H-16060)	5'-TCAGTTTTTGGTTTACAAGACCAATG-3'
12S	L1091	5'-AAACTGGGATTAGATACCCCACTAT-3'
	H1478	5'-GAGGGTGACGGGCGGTGTGT-3'
Phosducin	PHOF2	5'-AGATGAGCATGCAGGAGTATGA-3'
(PDC)	PHOR1	5'-TCCACATCCACAGCAAAAAACTCCT-3'

Table 3: The profile used for each gene.

	Cytochrome b	*12S*	*Phosducin*
Denaturation	94 ^{o}C 300 sec.	94 ^{o}C 90 sec.	95 ^{o}C 120 sec.
Annealing	94 ^{o}C 45 sec.	94 ^{o}C 45 sec.	94 ^{o}C 35 sec.
	47 ^{o}C 45 sec.	52 ^{o}C 45 sec.	50 ^{o}C 35 sec.
	70 ^{o}C 120 sec.	72 ^{o}C 90 sec.	72 ^{o}C 150 sec.
Extraction	72 ^{o}C 5 min.	72 ^{o}C 5 min.	72 ^{o}C 5 min.
Pause	8 ^{o}C ∞	8 ^{o}C ∞	8 ^{o}C ∞
No. of cycling	32 cycles	33 cycles	32 cycles

Equipment, Solutions and Chemicals:

The instruments used for laboratory analysis are listed in table 4. A list of chemicals, enzymes and other materials is given in table 5 and a list of buffers and solutions is given in table 6.

Table 4: The analytical instruments used in the present study.

Instruments	***Company***
ABI GeneAmp PCR System 9700	Applied Biosystems
Automated sequencer 3130XL	Applied Biosystems
Certified Thin wall 96 x 0.2 ml	Star Lab
Electronic Precision Balance U4100	Satorius
Electrophoresis power supply model 125	Biometra
Gel chambers for agarose gel: Agagel Standard	Biometra
Incubator & Shaker: Mixing Block MB-102	Biozym
Laboratory Parafilm	Roth
Magnetic drive RET	Janke & Kunkel
Micro-centrifuge tubes 1.5 ml	Star Lab
Micro-centrifuge: 5415D	Eppendorf
Micropipettes set (10, 20, 200, 1000 µl)	Eppendorf
Multipette Plus	Eppendorf

Continuation of table 4.

Instruments	*Company*
PCR tubes 0.2 ml, 8 strip	Biozym
PCR tubes with attached flat caps 0.2 ml	Star Lab
Systems analysis and gel documentation Bio-Vision + 3000. WL / 26 MX	PEQLAP
Thermocycler ABI GeneAmp 9700	Applied Biosystems
Vacuum Pump: N86 KN.18	KNF Neuberger
Vortex – 2 Genie 560E	BOHEMIA
Vortex REAX2000	Heidolph

Table 5: The chemicals, enzymes and solutions used in the present study.

Chemicals, Enzymes and other Materials	*Company*
Acetic acid	AppliChem
Agarose low EEO	AppliChem
BigDye 3.1	Applied Biosystems
ddH2O	Roth
EDTA	AppliChem
Ethanol absolute	Sigma-Aldrich

Continuation of table 5.

Chemicals, Enzymes and other Materials	*Company*
Ethidium bromide 1% (10 mg / ml)	Roth
Gene Ruler DNA ladder (100 – 1000 bp)	Fermentas
Gloves rotiprotict Latex	Roth
Gloves rotiprotict Nitril	Roth
GoTaq® green buffer	Promega
HiDye	Applied Biosystems
Isopropanol	ACROS ORGANIC
NaCl	AppliChem
Nucleotides	Promega
Primer	Operon
Proteinase K	Roth
Sodium dodecyl sulfate (SDS)	AppliChem
Sodium Acetate	
Taq DNA polymerase	Promega
Tris	Roth

Table 6: The buffer and solutions used in this study.

Stock Solutions	***Components***
Agarose gel solution	1.0% agarose, 1 µg/ml ethidium bromide, in water
Buffer PB	Guanidine hydrochloride, isopropanol
Buffer PE	10 mM Tris-HCl pH 7.5, 80 % ethanol
Extraction buffer	2 ml tris 1M (pH 8), 4 ml NaCl 5M, 4 ml EDTA 0.5M (pH 8), 190 ml ddH_2O steril.
Nucleotide mix	
TAE running Buffer (1 L. of 50 X)	242.0 g Tris-base, 57.1 ml Glecial Acetic Acid, 37.2 g EDTA, (pH 8.5), 1L dH_2O
Tris	1M (pH 8)

Purification

The PCR products were cleaned by using QIAquick PCR Purification Kit Protocol with some modification by adding 110 µl of buffer PB to 22 µl of each PCR product sample then mixing. A QIAquick spin column is placed in a 2 ml collection tube. After that, the sample is applied to the QIAquick column and centrifuged in 2 minutes with 13000 rpm to bind the DNA. Then discard the extracted solution. Add 450 µl of buffer PE (after adding ethanol (96-100%) to buffer PE) and centrifuge in 2 minutes with 13000 rpm centrifuge. Once again, discard the extracted solution. The QIAquick column is returned back into the same tube. To completely remove the residual ethanol from the buffer PE, centrifuge in 1.0 minute with 13000 rpm centrifuge again. The QIAquick column is placed in a clean 1.5 ml microcentrifuge tube. Add 30 µl H2O to the center of the QIAquick membrane and centrifuge the column for 1 min to release the DNA. The purified PCR product is checked on a gel electrophoresis as explained above.

Sequencing

The PCR products were sequenced directly on automated sequencers with the primers listed in table 3. The total volume for the subsequent sequencing reactions was 10 µL. 2 to 3 µL of cleaned PCR product were used with 0.5 µL BigDye 3.1 and 0.3 µM primer. Following an initial denaturation for 1 min at 96°C 25 cycles followed by 10 sec. at 96 °C, a 5 sec. annealing step at 50 °C and a 4 minutes extension at 60 °C. The PCR Products were cleaned by adding 1 µL of a solution containing 1.5 M Sodium Acetate and 250 mM EDTA (pH 8) and precipitated with a fourfold volume of 95% ethanol during a 45 min centrifugation step at 1500 rpm.

The dried samples were eluted with 10µL HiDye before run on an automated sequencer.

Data preparation

Sequences were edited and aligned using Clustal-W, as implemented in the program package Codon Code Aligner, ver. 2.0.6, and in MEGA 4.0 (Kumar et al., 2008). The sequences were carefully checked and corrected manually (to check for sequence errors) by using the print out of the sequence chromatographs. All sequences were compared with closely related taxa and populations.

Data analysis

Basic sequence statistics were obtained from the program PAUP* v. 4.0b10 (Swofford 2002) given that the various phylogenetic methods available often involve different assumptions about models of evolutionary change. The similarity of phylogenies produced by different methods increase confidence that the topologies involved are representative of the evolutionary history of the genes included. A Neighbor-Joining (NJ) tree based on uncorrected p-distances was constructed with MEGA vers. 4.0 (Kumar et al., 2008) and PAUP* v. 4.0b10 (Swofford 2002) in order to gain a first view of differentiation among sequences.

Phylogenetic analyses were performed using the programs PAUP* v. 4.0b10 (Swofford 2002), PHYLIP package (which is found on the website online program www.atgc-montpellier.fr/phyml/ with model parameters fitted to the data by likelihood maximization) and MrBayes, vers. 3.1.2 (Ronquist and Huelsenbeck 2003).

Bayesian and Maximum Likelihood (ML) were performed in order to check for consistency in the results using different algorithms based on different assumptions of molecular evolution. Bayesian inference phylogenetic analyses were conducted using MrBayes ver. 3.1.2 (Ronquist and Huelsenbeck 2003). All analyses began with a random starting tree, were run for 1,000,000 generations and were sampled every 100 generations for each independent mitochondrial genes (12S and cytochrome *b*), 2,000,000 generations for the combined data set (12S + cytochrome *b*) and 1,000,000 generations for the nuclear gene PDC. 'Burn in' trees (2500) discarding the first 25 % generations and the remaining samples were used to estimate the posterior probability values, branch length and topology. The Akaike Information Criterion (AIC) has been shown to have many advantages over the likelihood ratio test in selecting the best-fit model of nucleotide substitution (Posada and Buckley 2004). The AIC as implemented in MrModeltest ver. 2.3 (Nylander 2004) was used to estimate the best-fit model of nucleotide substitution for each data partition for each gene which were as the following:

1. 12S gene: nst = 6, Rates = gamma, and the model selected was GTR+G.
2. Cyt *b* gene: nst = 6, Rates = gamma, and the model selected was GTR+I+G.
3. Mitochondrial gene (cyt *b* + 12S genes): nst = 6, Rates = gamma, and the model selected was GTR+I+G.
4. PDC nuclear gene: nst = 2, Rates = gamma, and the model selected was K80+G. Genetic distances were calculated using PAUP* v. 4.0b10 (Swofford 2002). In all analysis, sequences of two specimens of species *Hemidactylus angulatus* were used as an outgroup.

B- Morphological Characters

The morphological analysis for Yemeni geckos was done depending on the results of the phylogenetic tree. The phylogenetic tree clearly distinguishes eight operational taxonomic units (OTUs) of Yemeni *Hemidactylus* taxa that found on the mainland, in addation to *Hemidactylus flaviviridis* (fig. 6). Specimens for each OTU was studied alone as a separate group.

The morphometric characters were taken with a caliper to the nearest 0.1 mm by using a Vernier ® ROYAL. Scales and scansors count were measured directly from the target by using binocular microscope. To insure correct interpretation and to facilitate the description of the taxa, the morphometric and meristic characters used in this study were defined as shown in table 7.

The monophyletic clade of *Hemidactylus flaviviridis* is not included in the other statistical analysis such as Discriminant Analysis (DA) and Principal Component Analysis (PCA), because this species is related to the Tropical Asian, and clearly distinguished from other species by their morphological features.

Table 7: The abbreviation symbols used in the morphological analysis.

Abbreviations	*Characters*	*Description*
SVL	**Length of the head & body**	Measures the distance from tip of snout to cloacal aperture.
LT	**Length of the tail**	Counts the distance from cloacal aperture to tip of the tail.
VS	**No. of ventral scales**	Counts the transverse row across the belly that includes the greatest number.
DS	**No. of dorsal scales**	Counts the mid-way scales between the fore and hind limbs.
TD	**Tubercle rows on dorsum**	Body tubercles are the conspicuously enlarged scales forming relatively straight longitudinal rows on the dorsal and lateral surfaces of the body. It is counted from the mid-body.
UL	**Upper labials**	Counts number of scales for one side starting from the angle of the mouth to the middle of upper jaw except rostral.
LL	**Lower labials**	Counts number of scales for one side starting from the angle of the mouth to the middle of lower jaw except mental.
In G	**Internasal granules**	Counts the scales between supranasal.
NsN	**Nasals surrounding nostril**	Counts the scales surrounding nostril.

Continuation of table 7.

Abbreviations	*Characters*	*Description*
1st Sc	**Scansors under 1st toe**	Counts the subdigital lamellae in a single row of scales from the base of toe to the tip of the 1st toe.
4th Sc	**Scansors under 4th toe**	Counts the subdigital lamellae in a single row of scales from the base of toe to the tip of the 4th toe.
MP	**Male pores**	Counts include the total number of femoral pores in both right and left rows of males; or of the pre-anal pores which are confined to the area in front of the vent.
HL	**Head length**	Measures the distance from tip of snout to the reteroarticular process of jaw.
HW	**Head width**	Measures the maximum width of head.
HH	**Head height**	Measures the maximum height of head, from occiput to underside of jaws.
OD	**Orbital diameter**	Measures the greatest diameter of orbit.
EED	**Eye to ear distance**	Measures the distance from anterior edge of ear opening to posterior corner of eye.
SED	**Snout to eye distance**	Measures the distance between anterior point of eye and tip of snout.

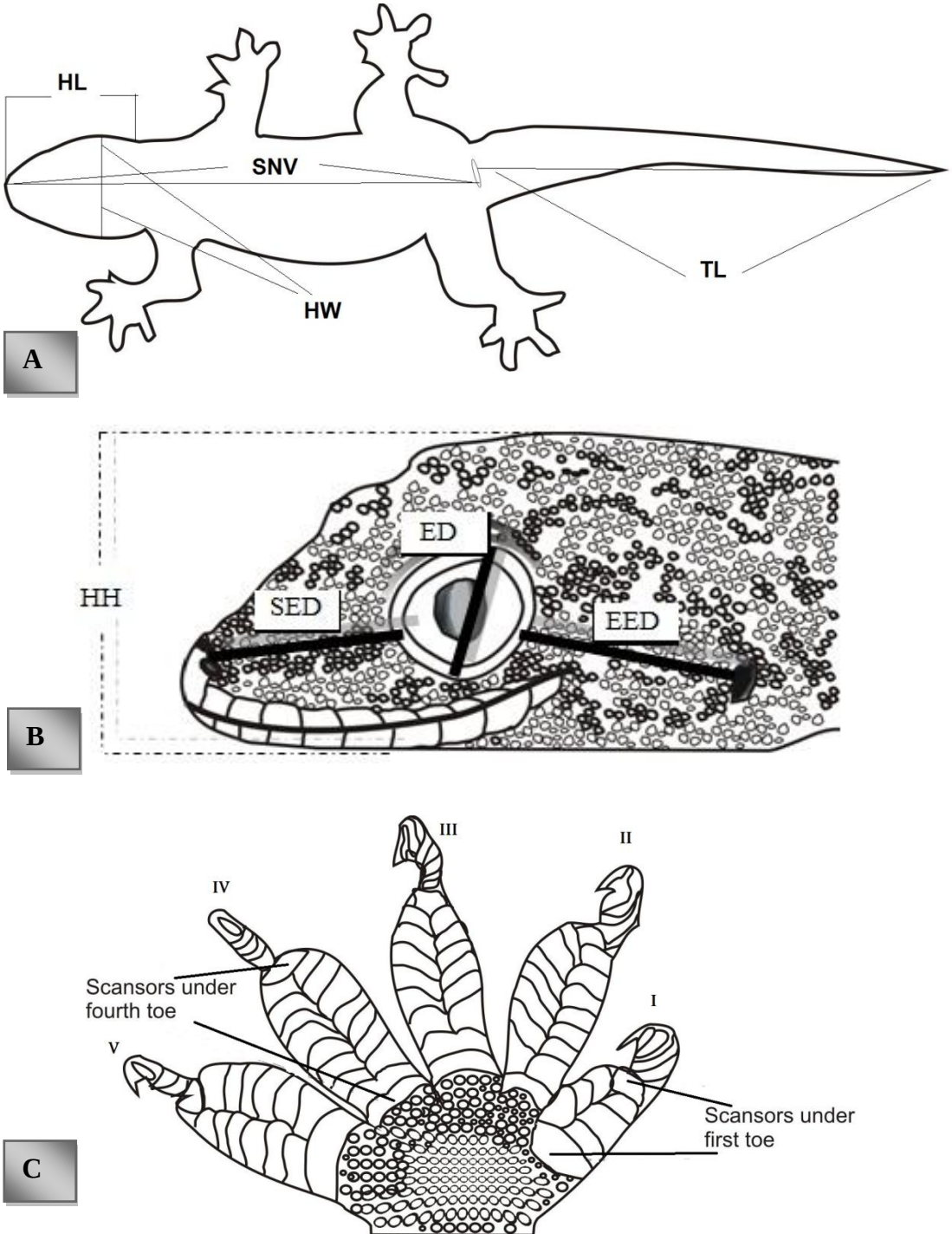


Figure 5: Morphological characteristics used for the identification of *Hemidactylus* species. A. Example of the snout to vent length (SVL) measurement. B. head length. C. Position and number of scansors from the hind feet.

The Statistic Analysis

Morphometric and meristic data were included in discriminant function analyses (DFA) and principal component analyses (PCA) using the analysis program of SPSS for windows, version 18.

Discriminant Function Analysis (DFA) was performed as a statistic analysis to generate a linear combination of variables that maximized the probability of correctly assigning observations to their pre-determined groups and to classify new observations into one of the groups. Factors with an eigenvalue over 1 were extracted for the principal component analyses (PCA). The first PCA was performed only with morphometric variables; in this analysis, the first principal component that largely corresponds to the size factor was excluded. In the second analysis the data analyzed were only the meristic data.

To assess significance of differences among taxa One-Way-ANOVA test and Independent-Samples T-test ($P < 0.05$) were performed. These results were confirmed by using the test of Mann-Whitney (U-test) $P < 0.05$. The analysis was performed only on females in the case of clade 4 and 8.

Results

A: DNA Barcoding and OTU Determination

The partial mitochondrial gene of 12S was chosen since it can be amplified successfully and the required comparative data are available in Genbank. One hundred and eighty five Yemeni *Hemidactylus* specimens were sequenced, in addition to data collected from the Genbank from several representative taxa based on the study of Carranza and Arnold (2006) from Yemen and neighboring countries. Furthermore, tissues of known species of *Hemidactylus* from Socotra Archipelago collected and identified by Prof. Dr. U. Joger were sequenced within the study of the previous specimens (12S rRNA gene). These species are: *H. forbesii*, *H. granti*, *H. homoeolepis*, *H. oxyrhinus* and *H. turcicus* (table 9).

The operational taxonomic units (OTUs) were defined as monophyletic clades in the mitochondrial genetic trees (fig. 6).

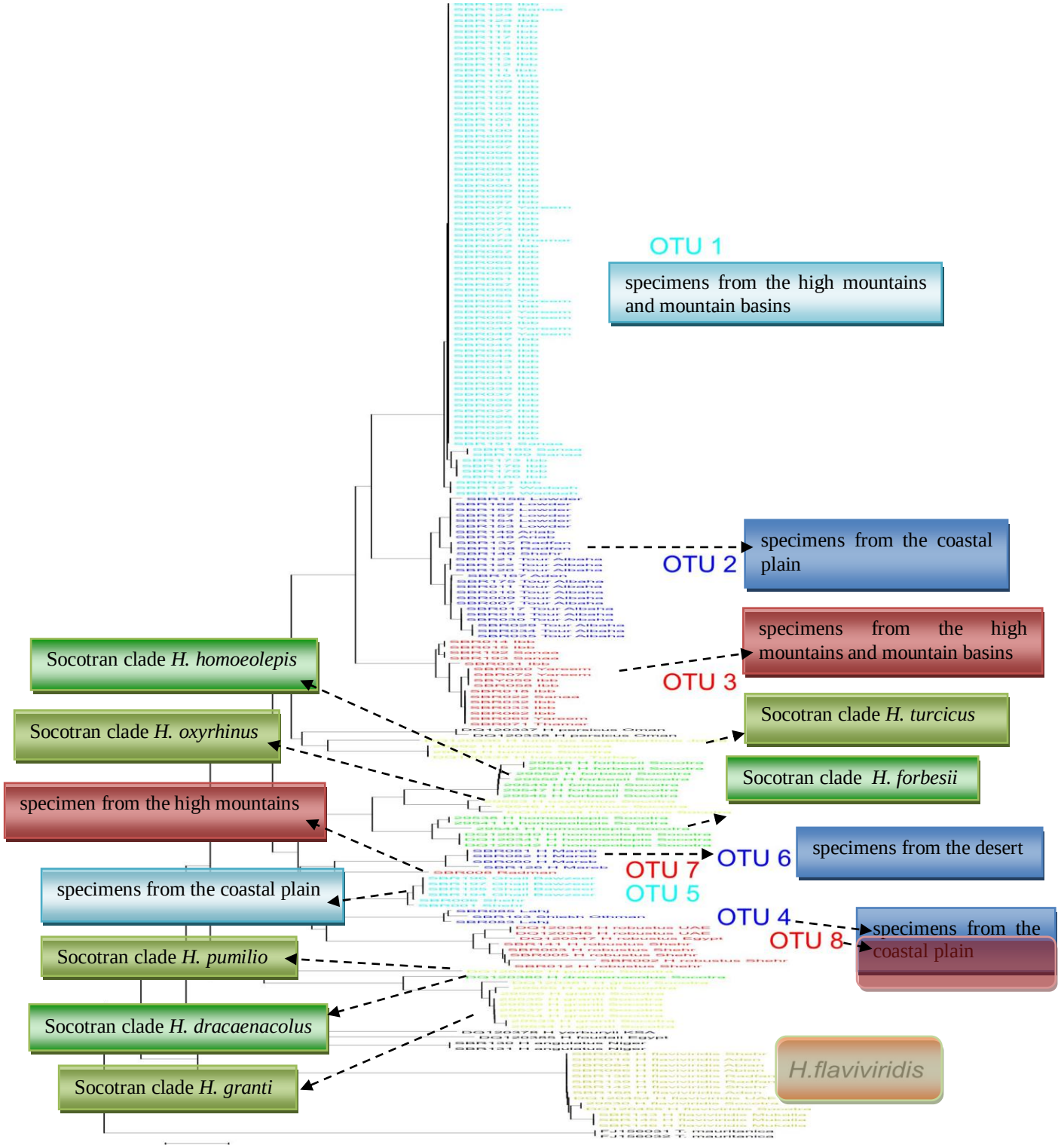


Figure 6: 12S tree, Neighbor-Joining (NJ), obtained from MEGA. The colored clades represent Yemeni *Hemidactylus* sequences. The range of green is confined to the Socotran specimens. The clade of *H. flaviviridis* contains specimens from the mainland and Socotra Island.

B: Phylogenetic analysis

As a result of 1465 characters for the 12S rRNA, cytochrome *b* and Phosducin (PDC) a molecular phylogeny was obtained of the geckos collected (370 bp of the 12S and 736 bp of the cytochrome *b* as mitochondrial genes and 359 bp of the PDC as a nuclear gene).

Some sequences were selected from each Yemeni clade which are representatives of all major clades presented in the neighbor-joining approach for further analysis.

Maximum Likelihood (ML) and Bayesian analysis for genes which were applied in this study gave very similar results and showed only slight differences at the base of the tree where their relationships have a small support value.

Throughout the study, samples of *Hemidactylus angulatus* were used to root all the gene trees, since this species does not belong to the ingroup and is not too far from the ingroup. Furthermore, it is not a part of the 'arid clade' sensu Carranza and Arnold (2006).

In all analyses of mitochondrial genes, all Yemeni clades have a very strong bootstrapping value in ML and Bayesian support except the clade of OTU 6 and OTU 7 in the tree of cytochrome *b* gene.

The results of the phylogenetic trees revealed several main groups of *Hemidactylus* in Yemen. Each group consists of a number of OTUs from the mainland or Socotra Archipelago (fig. 7-10).

Table 8: Sequenced specimens and their reference numbers of *Hemidactylus* that are presented in the results in this study.

*Code **	*Species***	*Clade*	*Locality*	
N41810	*H. yerburii* ssp.	1	Ibb	13° 58` N - 44° 11` E
N41854	*H. yerburii* ssp.	1	Sana'a	15° 23` N - 44° 14` E
N41856	*H. yerburii*	2	Tour Al-Baha	12° 58` N - 44° 53` E
N41883	*H. yerburii*	2	Lowder	13° 52` N - 45° 55` E
N41892	*H.* sp.	3	Sana'a	15° 23` N - 44° 14` E
N41890	*H.* sp.	3	Ibb	13° 58` N - 44° 11` E
N41902	*H. sinaitus*	4	Lahj	13° 00` N - 45° 54` E
N41904	*H. sinaitus*	4	Shaikh Othman	12° 55` N - 44° 59` E
N41908	*H.* sp.	5	Ash-Shihr	14° 45` N - 49° 36` E
N41911	*H.* sp.	5	Ghail Bawzeer	14° 47` N - 49° 22` E
N41912	*H.* sp.	6	Mareb	15° 27` N - 45° 20` E
N41913	*H.* sp.	6	Mareb	15° 27` N - 45° 20` E
N41916	*H.* sp.	7	Radman	Not located
N41918	*H. robustus*	8	Ash-Shihr	14° 45` N - 49° 36` E
N42044	*H. robustus*	8	Ash-Shihr	14° 45` N - 49° 36` E

* Codes refer to voucher specimens.
** Identification of species is done depending on several criteria. Some species are collected from the type locality, which the description of their characters fit to the diagnosis of species, as well they were compared with specimens from other museums (for more details see the discussion).

Table 9: Sequenced samples from Socotra archipelago and Genbank samples and their reference numbers of *Hemidactylus* used in this study.

Species	***Locality***	***Genbank***		
		Cyt b	***12S***	***PDC***
H. angulatus (2)	Nigeria	EU268399		EU268336
H. citernii (3)	Somalia	DQ120212	DQ120383	-
H. citernii (3)	Somalia	DQ120213	DQ120384	-
H. dracaenacolus (3)	Socotra island	DQ120209	DQ120380	-
H. forbesii (3)	Abd el Kuri	DQ120168	DQ120339	-
H. forbesii (1)	Abd el Kuri	-	N29550	-
H. forbesii (1)	Abd el Kuri	-	N29551	-
H. foudaii (3)	Egypt	DQ120214	DQ120385	-
H. granti (3)	Socotra island	DQ120210	DQ120381	-
H. homoeolepis (3)	Socotra island	DQ120169	DQ120340	-
H. homoeolepis (3)	Socotra island	DQ120170	DQ120341	-
H. homoeolepis (1)	Socotra island	-	N29539	-
H. homoeolepis (1)	Socotra island	-	N29540	-
H. oxyrhinus (3)	Abd al Kuri	DQ120173	DQ120344	-
H. oxyrhinus (1)	Abd al Kuri	-	N29552	-
H. persicus (3)	Oman	DQ120166-7	DQ120337-8	-

Continuation to Table 9

H. persicus [2]	Pakistan	EU268409	-	EU268346
H. pumilio [3]	Socotra island	DQ120211	DQ120382	-
H. robustus [3]	UAE	DQ120175	DQ120346	-
H. robustus [3]	Egypt	DQ120176	DQ120347	-
H. robustus [2]	Pakistan	EU268408	-	EU268345
H. t. lavadeserticus [3]	Jordan	DQ120165	DQ120336	-
H. turcicus [1]	Socotra	-	N29592	-
H. turcicus [1]	Socotra	-	N29593	-
H. turcicus [2]	USA	EU268392	-	EU268329
H. turcicus [3]	Turkey	DQ120163	DQ120334	-
H. yerburii ? [3]	KSA	DQ120207	DQ120378	-

References:

1. From the collection samples of Natural History Museum, Braunschweig, Germany (NHM-BS).
2. From Bauer, et al. 2008.
3. From Carranza and Arnold (2006).

Result of cytochrome *b* gene:

For the cytochrome *b* gene fragment, the analysis of the tree revealed that the basal dichotomy separates a clade consisting of northeast African *H. foudaii* from Egypt and the east African *Hemidactylus citernii* from Somalia from a unit comprising all other Arabian members of the genus *Hemidactylus*. The ML tree identified five Yemeni main groups, three groups from the mainland and the remaining from the Socotra Archipelago (fig. 7):

The first group is the clades of the Socotran species *H. granti* and *H. dracaenacolus* that form a basal clade of the Arabian lineages with 95 % bootstrap value in ML analysis and 100 % Bayesian support.

The second group consists of the clades of OTU 1 (from the high mountains and mountain basins of Yemen), OTU 2 (from the coastal plain and plateaus of Yemen) and OTU 3 (from the high mountains and mountain basins) with 98 % Bayesian support and 88 % bootstrap value in ML. Moreover, the clade of OTU 1 and OTU 2 is well supported by bootstrap value 96 % in ML analysis and 100 % Bayesian support. These three clades are a sister to clades composed of *H. pumilio* (DQ120211) identified in Genbank (Carranza and Arnold 2006) from Socotra and *H. persicus* (DQ120166-7) from Oman. The single sequence of *H. pumilio* was a sister to the member clade of *H. persicus* with significant support value (85 %) in ML and (93 %) in Bayesian analysis.

The third group consists of the clades of OTU 4 (from the coastal plain) and the clade of *H. robustus* (DQ120176, DQ120174) from Egypt and United Arab Emirates and other samples collected in this study (OTU 8) as well from the coastal plain. *H.*

robustus is a sister to the clade of OTU 4 with strong bootstrapping value in ML (94%). On the contrary, it has a weak Bayesian support (82 %).

The fourth group is another Socotran group composed of *H. oxyrhinus* (DQ120173) from Abd Al-Kuri Island (Socotra Archipelago) and *H. homoeolepis* (DQ120169-DQ1201971) from Socotra Island with relatively good bootstrap value (74 %) in ML analysis. However, with low Bayesian support (pp < 90 %).

The fifth group comprises the clades of OTU 5 (from the coastal plain), OTU 6 (from the desert) and a single sequence of OTU 7 (from the high mountains) with 97% Bayesian support and 80 % bootstrap value in ML analysis. The genetic divergence between the monophyletic clades of OTU 5, 6 and 7 was 13 – 16 %. Within this group, a sequence of *Hemidactylus* identified in Genbank (Carranza and Arnold 2006) as *H. yerburii* (DQ120207) from south of Saudi Arabia was aligned with weak bootstrapping value in both analysis of Bayesian and ML.

The clade of *H. turcicus* (DQ120163, DQ120165) had a relatively good bootstrapping value with 87 % in the ML analysis and 99 % in the Bayesian analysis. This clade is a sister to the groups 3, 4 and 5.

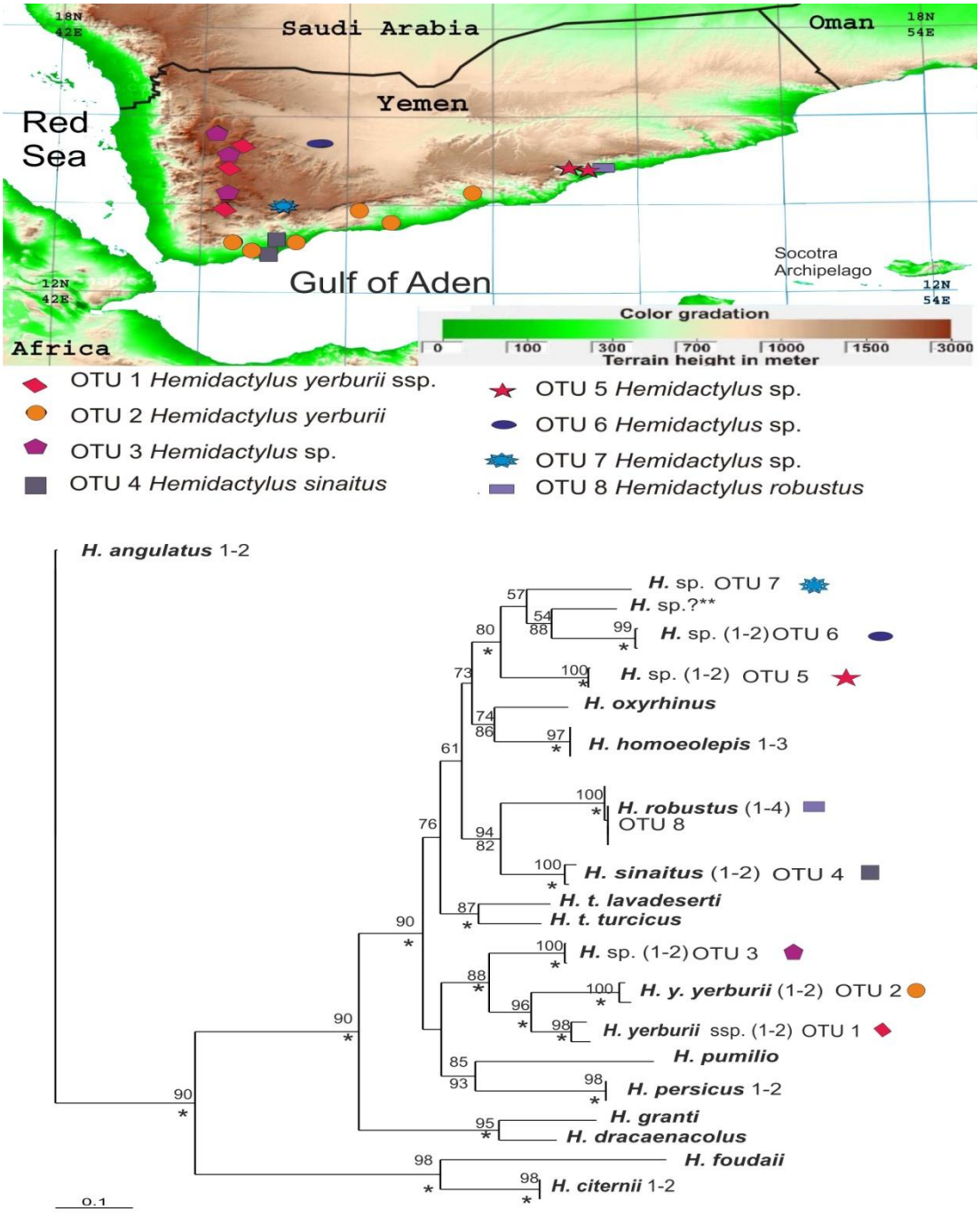


Figure 7: (above) Distribution of mitochondorial lineages of *Hemidactylus* in the mainland of Yemen.

(below) The ML tree for the cytochrome *b* mtDNA sequences obtained with PHYML. Numbers by the nodes indicate: for ML bootstrap values (> 50%) are given above the nodes, and Bayesian probabilities are given below the nodes. An asterisk (*) indicates a posterior probability of ≥ 0.95.

**** A sequence of *Hemidactylus* from Najran, Saudi Arabia, identified in Genbank as *H. yerburii*.**

***** The numbers between brackets refer to the samples in table 8 and 9.**

Result of 12S rRNA gene:

For the 12S rRNA gene fragment, the analysis of the tree of this gene reveals that the African clades of *H. citernii* and *H. foudaii* were a sister to the Arabian members of the genus. A single sequence of *Hemidactylus* identified in Genbank as *H. yerburii* (Carranza and Arnold 2006) aligned with the group of *Hemidactylus foudaii* and *H. citernii* with bootstrap support value of 96 % in Bayesian analysis and 82 % bootstrap value in ML.

The analysis of the ML tree identified six Yemeni groups; three of them are from the mainland and the other are from the Socotra Archipelago (fig. 8):

The first group consists of the clades of the Socotran species *H. dracaenacolus* and *H. granti* that form a clade with 94 % bootstrap value in ML analysis and 100 % Bayesian support. The single sequence of *H. pumilio* is a sister group of *H. dracaenacolus* and *H. granti* with a weak bootstrap value in ML analysis.

The clade of *H. persicus* had a good bootstrap value with 97 % in ML analysis and it forms a sister for all clades except the clades of the first group. In Bayesian analysis, this clade had a strong Bayesian support with 100 %.

The second group is the clade of *H. t. turcicus* from Turkey and Socotra and *H. turcicus lavadeserticus* from Jordan with 72 % bootstrap value in ML analysis. However, with low supported value in the Bayesian analysis.

The third group comprised the mainland clades of OTU 1, OTU 2 and OTU 3 with 100 % Bayesian support and 97 % bootstrap value in ML analysis. The clade of

OTU1 and OTU 2 is very well supported by bootstrap value 98 % in ML analysis and 100% Bayesian support.

The fourth group consists of the mainland clades of OTU 4 and *H. robustus* and other samples collected in this study (OTU 8). *H. robustus* is a sister group to OTU 4 with 100 % Bayesian support and 92 % bootstrap value in ML. The genetic distance between these two clades was 10 %.

The fifth group is the mainland clades of OTU 5, OTU 6 and a single sequence of OTU 7. This group had a low support in both analysis in ML and Bayesian. However, the sequence of OTU 7 was a sister of OTU 6 with 100 % Bayesian support and 92 % bootstrap value in ML analysis.

The last group comprises *H. oxyrhinus, H. forbesii* and *H. homoeolepis.* The latter was the sister to other two with 100 % Bayesian support and 96 % bootstrap value in ML analysis. The clade of *H. oxyrhinus, H. forbesii* has 91 % Bayesian support, however, a weak supported clade (pp < 50 %) in ML analysis.

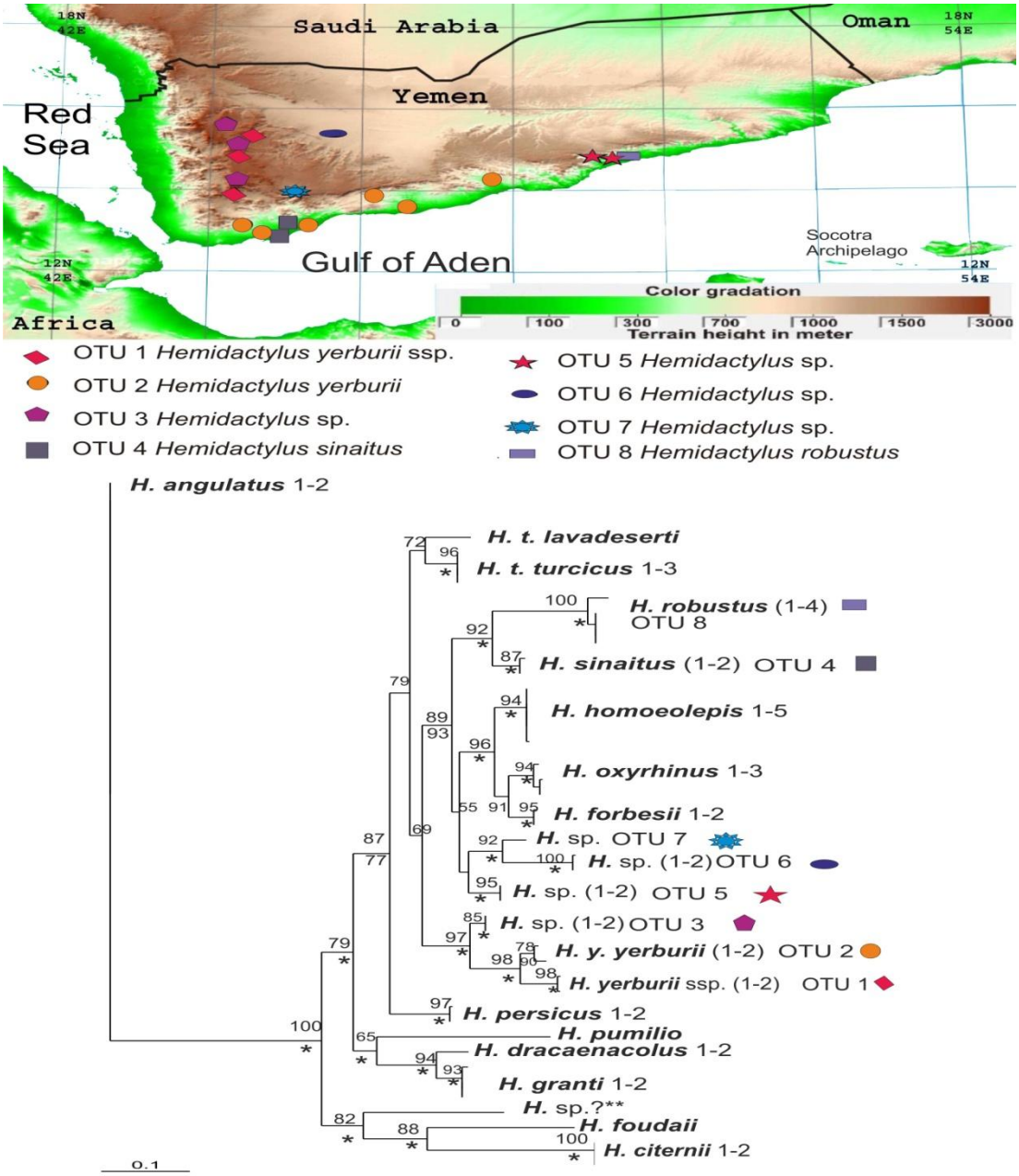


Figure 8: (above) Distribution of mitochondorial lineages of *Hemidactylus* in the mainland of Yemen.

(below) The ML tree for the 12S rRNA mtDNA sequences obtained with PHYML. Numbers by the nodes indicate: for ML bootstrap values (> 50%) are given above the nodes, and Bayesian probabilities are given below the nodes. An asterisk (*) indicates a posterior probability of ≥ 0.95.

**** A sequence of *Hemidactylus* from Najran, Saudi Arabia, identified in Genbank as *H. yerburii*.**

***** The numbers between brackets refer to the samples in table 8 and 9.**

Result of combined mitochondrial gene:

In the combined mitochondrial gene fragments (1106 bp from cyt *b* and 12S), the African clades of *Hemidactylus citernii* and *H. foudaii* was a sister group to the Arabian members of the genus. The analysis of the ML tree reveals six Yemeni main groups. Three groups are from the mainland and the other from the Socotra Archipelago. All Yemeni clades have a very strong bootstrapping value in both analysis of ML and Bayesian, except the clade of OTU 6 and OTU 7 (fig. 9):

The first group is the clade of the Socotran species *H. granti* and *H. dracaenacolus* that form a basal clade of the Arabian lineages with 100 % bootstrap value in ML analysis and 100 % Bayesian support.

The second group is formed by Socotran species *H. pumilio* which is a sister to all ingroup members of *Hemidactylus* except the clades of *H. citernii*, *H. foudaii*, and the first group. This inclusive clade has a supported value of 72 % in the ML analysis. On the contrary in Bayesian analyses, *H. pumilio* was a sister to the clade of *H. persicus* with a low supporting value.

The third group consists of the mainland clades of OTU 1, OTU 2 and OTU 3 with 100 % Bayesian support and 99 % bootstrap value in ML analysis. The clade of OTU3 is a sister to OTU 1 and OTU 2 with strong supported value in both analyses.

The fourth group consists of the mainland clades of OTU 4, OTU 8 and *H. robustus*. This group had a strong bootstrapping value of 98 % in ML analysis as well as a strong Bayesian support clade 100 %.

The fifth group comprises the Socotran Archipelago species *H. oxyrhinus* and *H. homoeolepis.* The latter was the sister to the former with 100 % Bayesian support and 93 % bootstrap value in ML analysis.

The sixth group comprises the mainland clades of OTU 5, OTU 6 and a single sequence of OTU 7 with a good support value (90 %) in ML analysis. However, in the Bayesian analysis this group had a weak supported value. The clade of OTU 5 is a sister to the clades of OTU 6 and OTU 7. The sequence of OTU 7 is a sister of OTU 6 with 72 % bootstrap value in ML. But in Bayesian analysis it has a weak support.

The single sequence of *H. yerburii,* identified in Genbank, aligned as a sister to the clades of OTU 6 and OTU 7 with low Bayesian support (71 %). However, in ML analysis this sequence of *H. yerburii* itself separated as a sister group to all groups except the first and second groups.

The clade of *H. turcicus* has a strong bootstrapping value with 87 % in ML and 99 % Bayesian support. The clade of *H. persicus* has a very strong bootstrapping value with 100 % in both analysis. However, no Yemeni specimens aligned with these clades.

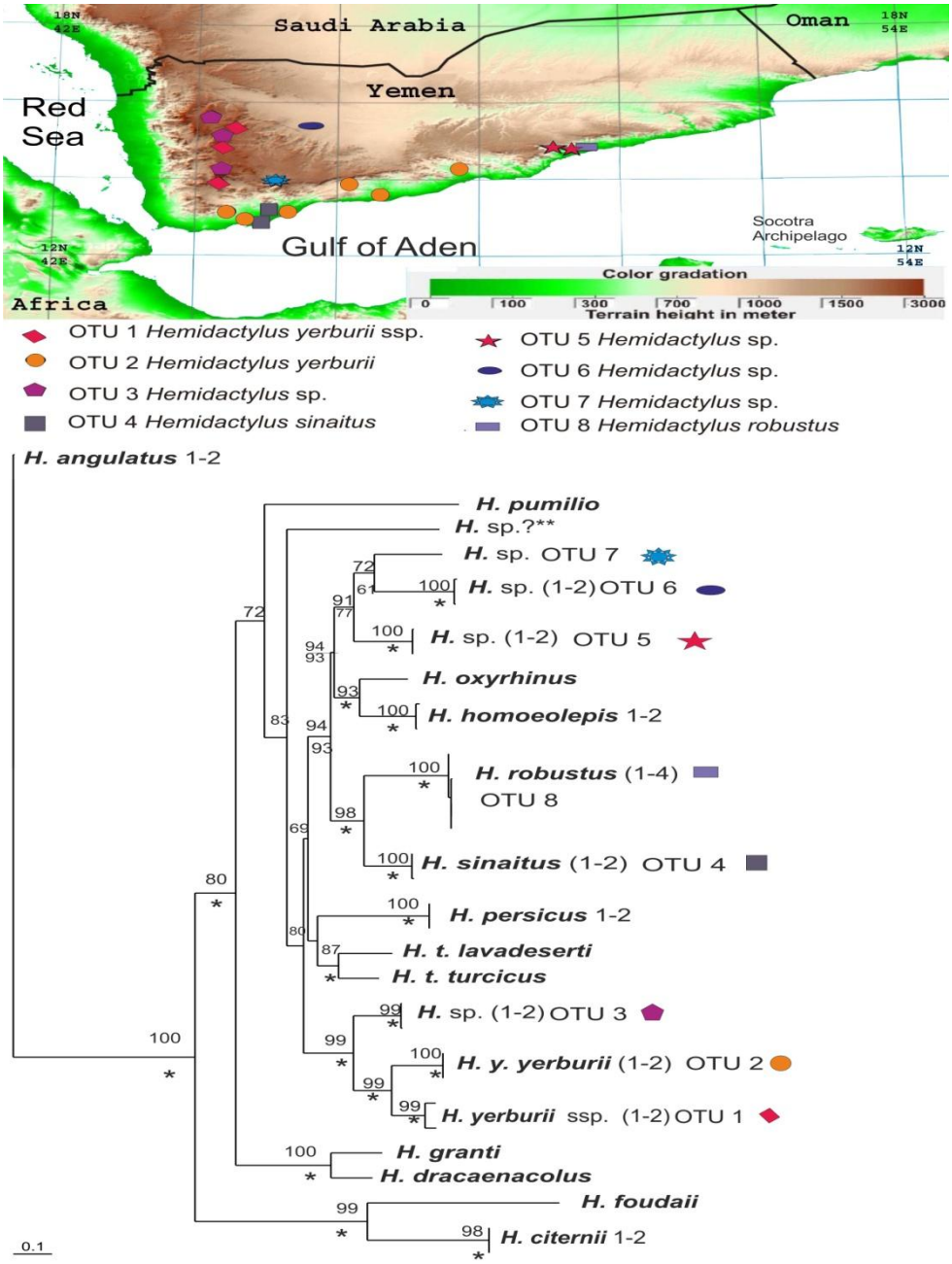


Figure 9: (above) Distribution of mitochondorial lineages of *Hemidactylus* in the mainland of Yemen. (below) The ML tree for a combination of the cytochrome *b* and 12S rRNA mtDNA sequences obtained with PHYML. Numbers by the nodes indicate: for ML bootstrap values (> 50%) are given above the nodes, and Bayesian probabilities are given below the nodes. An asterisk (*) indicates a posterior probability of ≥ 0.95.

**** A sequence of *Hemidactylus* from Najran, Saudi Arabia, identified in Genbank as *H. yerburii*.**

***** The numbers between brackets refer to the samples in table 8 and 9.**

Result of the nuclear gene (PDC):

The molecular phylogeny of *Hemidactylus* in 359 base pairs of the nuclear gene Phosducin (PDC) distinguished four groups of the clades of Yemeni geckos. The samples used in this gene were collected from the mainland in addition to three sequences for known species from the Genbank, based on the study of (Bauer et al., 2008). *Hemidactylus angulatus* from Niger was used to root the tree (fig. 10).

The first group comprises of the clade of OTU 3 with a very strong supporting value 100 % in Bayesian analysis and 93 % bootstrap value in ML analysis. The remaining clades do present a monophylum with regard to the first group (except OTU 1 and OTU 2 and *H. turcicus*). The sequences of the OTU 1, OTU 2 and *H. turcicus* are not supported as clades in the ML and Bayesian analysis.

The second group is the clade of OTU 6 with supported value of 82 % in ML analysis and 93 % in the Bayesian analysis.

The third group consists of the clade of OTU 5. The supported value in the Bayesian analysis is strong with 94 % and 87 % in the ML analysis.

The fourth group is the clade of OTU 4 and OTU 8 and the sequence of OTU 7 with a very high supported value of 93 % in ML analysis and 100 % in Bayesian analysis. The clade of *H. robustus* and the other samples collected throughout the study (OTU 8) is a sister to the clade of OTU 4 with a strong bootstrapping value (95 %) in ML and (96 %) in Bayesian analysis.

The OTU 4, 5, 6, 7 and 8 form good distinct clades with very well bootstrapping support as well the clade of OTU 3.

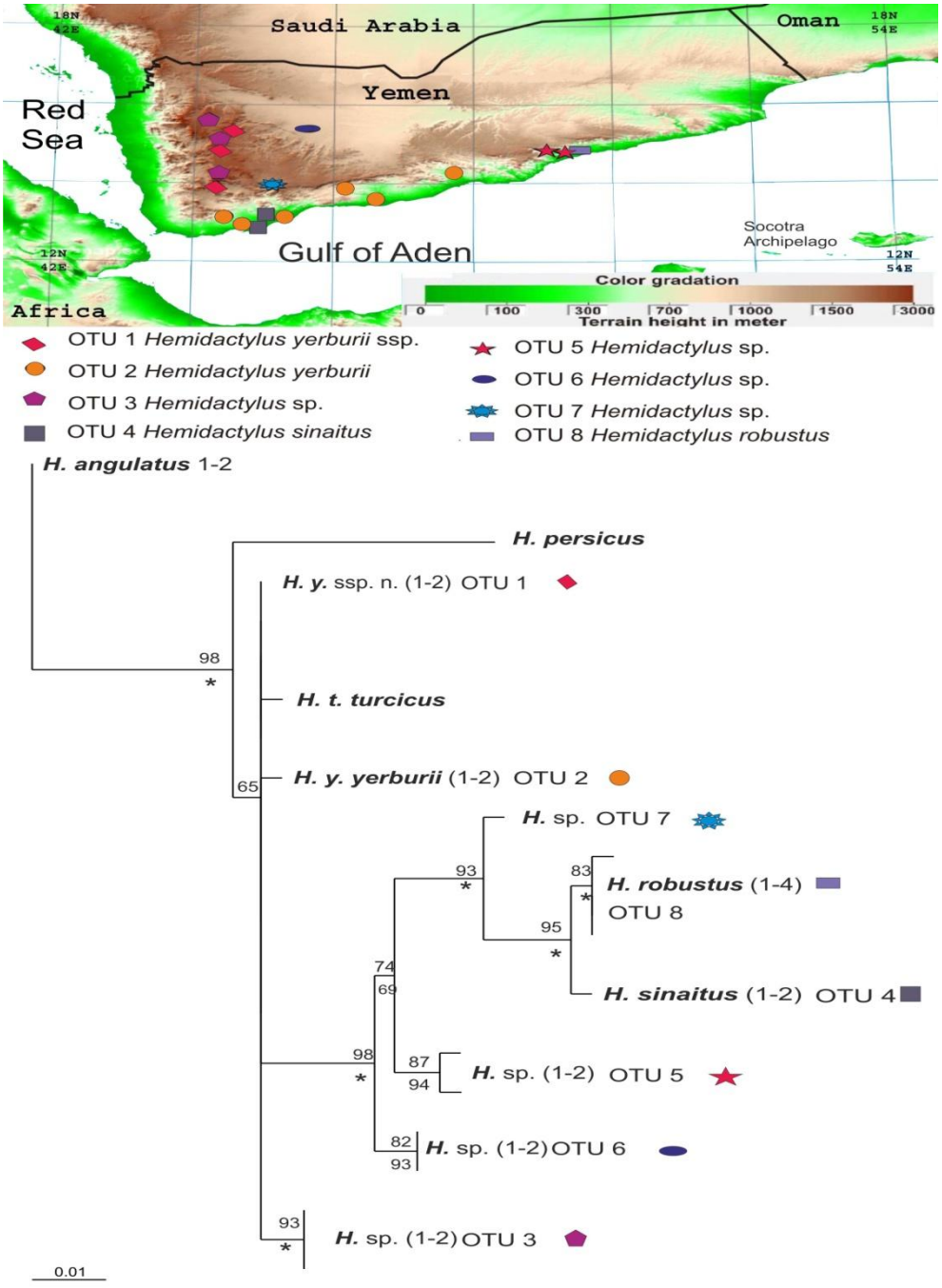


Figure 10: (above) Distribution of mitochondorial lineages of *Hemidactylus* in the mainland of Yemen. (below) The ML tree for the PDC nuclear gene sequences obtained with PHYML. Numbers by the nodes indicate: for ML bootstrap values (> 50%) are given above the nodes, and Bayesian probabilities are given below the nodes. An asterisk indicates a posterior probability of ≥ 0.95.

**** The numbers between brackets refer to the samples in table 8 and 9.**

C: Morphological Results

Results of examining the phylogenetic tree revealed eight monophyletic operational taxonomic units (OTUs) of *Hemidactylus* in the mainland of Yemen. These OTUs were compared with known species of *Hemidactylus* (Museum specimens and literature data).

Two clades are from the known species *H. yerburii* (OTU 1 and OTU 2), one clade (OTU 4) assigned as *H. sinaitus* and one clade is from the species of *H. robustus* (OTU 8).

The mean, standard deviation, minimum and maximum values of morphometric and meristic characters obtained for Yemeni geckos are shown in table 10 and 11.

The morphometric characters TL (tail length) and TT (tubercles on tail) are excluded, since the tail was unavailable (broken) in many samples.

For all statistical tests, relative measures were taken for morphometric characters (HL, HW, HH, OD, EED and SED) by dividing these characters by SVL.

Table 10: Mean values and standard deviation of different meristic characters for each Yemeni *Hemidactylus* clade. (n= number of specimens, for other abbreviations, see table 7).

1-* (OTU 1) *H. cf. yerburii	***VS***	***DS***	***TD***	***UL***	***LL***	***In G***	***1st Sc***	***4th Sc***	***MP***
N = 32 males									
Mean	40,91	87,19	15,09	10,31	7,94	1,00	6,25	10,19	10,19
Standard deviation	3,897	5,562	1,027	,644	,619	,000	,440	,397	1,330
Minimum	36	74	13	9	7	1	6	10	7
Maximum	48	97	16	12	9	1	7	11	12
N = 60 females									
Mean	42,45	85,40	15,47	10,37	7,83	1,00	6,25	10,10	0
Standard deviation	3,442	7,230	,873	,637	,526	,000	,508	,573	
Minimum	36	74	14	9	7	1	5	9	
Maximum	48	97	16	12	9	1	7	11	
2- (OTU 2) *H. y. yerburii*									
N = 10 males									
Mean	41,00	91,70	15,40	10,40	7,80	1,00	6,70	10,30	12,50
Standard deviation	5,657	4,423	,966	,516	,422	,000	,483	,483	1,958
Minimum	32	86	14	10	7	1	6	10	10
Maximum	50	99	16	11	8	1	7	11	17
N = 23 females									
Mean	41,35	91,30	15,30	10,48	7,91	,91	6,83	10,35	0
Deviation	4,468	6,116	,974	,790	,596	,288	,388	,487	
Minimum	34	82	14	10	7	0	6	10	
Maximum	48	102	16	12	9	1	7	11	

Continuation to table 10

***3- H. sp.* (OTU 3)**									
N = 2 males									
Mean	38,00	65,50	12,00	9,50	8,00	,50	7,00	11,00	7,50
Standard deviation	5,657	,707	,000	,707	,000	,707	,000	,000	2,121
Minimum	34	65	12	9	8	0	7	11	6
Maximum	42	66	12	10	8	1	7	11	9
N = 12 females									
Mean	35,50	67,08	12,75	10,00	8,08	,75	6,33	10,17	0
Standard deviation	2,541	5,195	1,545	,603	,669	,452	,492	,718	
Minimum	33	62	10	9	7	0	6	9	
Maximum	42	77	16	11	9	1	7	11	
4- (OTUs 4)									
N = 1 male									
Mean	34,00	68,00	14,00	8,00	8,00	1,00	5,00	10,00	7,00
Minimum	34	68	14	8	8	1	5	10	7
Maximum	34	68	14	8	8	1	5	10	7
N = 5 females									
Mean	35,00	73,00	14,20	8,80	7,00	1,00	5,00	9,00	0
Standard deviation	2,828	2,915	,447	,447	,000	,000	,000	,000	
Minimum	32	70	14	8	7	1	5	9	
Maximum	38	77	15	9	7	1	5	9	

Continuation to table 10

5- (OTU 5)									
N = 2 males									
Mean	52,50	78,00	13,00	9,50	8,00	1,00	6,00	10,00	6,00
Standard deviation	2,121	5,657	1,414	,707	,000	,000	,000	,000	,000
Minimum	51	74	12	9	8	1	6	10	6
Maximum	54	82	14	10	8	1	6	10	6
N = 2 females									
Mean	47,50	70,00	14,00	9,50	7,50	1,00	6,00	10,00	0
Standard deviation	2,121	1,414	,000	,707	,707	,000	,000	,000	
Minimum	46	69	14	9	7	1	6	10	
Maximum	49	71	14	10	8	1	6	10	
6- (OTU 6)									
N = 2 males									
Mean	31,00	76,50	14,00	8,50	8,00	1,00	8,00	11,00	6,00
Standard Deviation	1,414	,707	,000	,707	,000	,000	,000	,000	,000
Minimum	30	76	14	8	8	1	8	11	6
Maximum	32	77	14	9	8	1	8	11	6
N = 2 females									
Mean	30,00	80,00	14,00	9,00	8,00	1,00	8,00	11,00	0
Standard deviation	2,828	2,828	,000	,000	,000	,000	,000	,000	
Minimum	28	78	14	9	8	1	8	11	
Maximum	32	82	14	9	8	1	8	11	

Continuation to table 10

7- (OTU 7)	***VS***	***DS***	***TD***	***UL***	***LL***	***In G***	***1st Sc***	***4th Sc***	***MP***
N = 1 female									
Mean	41,00	63,00	14,00	8,00	8,00	1,00	5,00	8,00	0
Minimum	41	63	14	8	8	1	5	8	
Maximum	41	63	14	8	8	1	5	8	
8- (OTU 8) *H. robustus*									
N = 5 females									
Mean	37,80	70,80	15,60	8,60	7,40	,80	6,20	9,80	0
Standard deviation	1,304	5,805	,894	,548	,548	,447	,447	,447	
Minimum	36	61	14	8	7	0	6	9	
Maximum	39	75	16	9	8	1	7	10	
9- *H. flaviviridis*									
N = 6 males									
Mean	38,83	92,50	,00	12,33	10,33	1,00	8,67	12,67	13,00
Standard deviation	2,041	4,680	,000	1,033	,516	,000	,516	,516	2,191
Minimum	36	86	0	11	10	1	8	12	10
Maximum	42	98	0	13	11	1	9	13	16
N = 14 females									
Mean	38,43	91,14	,00	12,93	10,36	1,00	8,50	12,29	0
Standard deviation	2,209	5,157	,000	1,072	,633	,000	,519	,611	
Minimum	35	84	0	11	9	1	8	12	
Maximum	43	101	0	14	11	1	9	14	

Table 11: Mean values and standard deviation of different morphometric characters for each Yemeni *Hemidactylus* clade. (n= number of specimens, for other abbreviations, see table 7).

1- (OTU 1) H. cf. yerburii	***SVL***	***HL***	***HW***	***HH***	***OD***	***EED***	***SED***
N = 32 males							
Mean	51,038	16,641	11,481	6,719	3,622	4,781	6,772
Standard	6,5881	1,7731	1,3180	,8267	,4125	,5916	,7122
Minimum	39,2	13,4	8,6	5,1	3,1	3,6	5,0
Maximum	67,7	19,8	14,0	8,7	4,4	6,2	7,9
N = 60 females							
Mean	43,408	14,173	9,308	5,687	3,287	3,920	5,753
Standard deviation	9,4980	2,6585	1,8687	1,1037	,5404	,7133	1,1382
Minimum	22,9	8,4	4,9	2,8	2,0	2,3	2,9
Maximum	64,1	19,3	12,9	8,2	4,4	5,1	7,9
2- (OTU 2) *H. yerburii*							
N = 10 males							
Mean	55,990	18,230	12,600	7,680	3,920	4,980	7,530
Standard deviation	6,3060	2,1066	1,5677	,9852	,2821	,6070	,8629
Minimum	47,3	15,2	10,8	6,2	3,6	4,3	6,6
Maximum	64,4	21,3	15,6	9,5	4,4	6,3	9,2
N = 23 females							
Mean	50,478	15,943	10,530	6,517	3,709	4,457	6,904
Standard deviation	8,5522	2,0487	1,6069	,9119	,4316	,5599	,9749
Minimum	35,3	11,7	7,4	4,5	2,7	3,3	4,6
Maximum	61,5	18,7	12,8	8,1	4,3	5,1	8,0

Continuation to table 11

3- (OTU 3)	SVL	HL	HW	HH	OD	EED	SED
N = 2 males							
Mean	45,400	15,750	10,650	5,350	3,300	4,350	6,100
Standard deviation	,4243	,2121	,6364	,0707	,2828	,0707	,4243
Minimum	45,1	15,6	10,2	5,3	3,1	4,3	5,8
Maximum	45,7	15,9	11,1	5,4	3,5	4,4	6,4
N = 12 females							
Mean	41,367	13,567	8,933	5,008	3,042	4,100	5,242
Standard deviation	5,9624	1,7401	1,4202	,9977	,2999	,6194	,6529
Minimum	28,6	9,9	5,9	3,3	2,5	3,0	4,0
Maximum	47,6	15,6	10,5	6,6	3,6	4,9	6,2
4- (OTUs 4)							
N = 1 male							
Mean	37,000	10,500	6,800	5,100	2,500	3,400	4,200
Minimum	37,0	10,5	6,8	5,1	2,5	3,4	4,2
Maximum	37,0	10,5	6,8	5,1	2,5	3,4	4,2
N = 5 females							
Mean	29,440	9,500	5,820	4,020	2,320	2,940	3,700
Standard deviation	6,4833	1,1358	,8899	,8167	,3114	,4615	,4583
Minimum	22,5	8,3	4,9	3,3	2,0	2,5	3,4
Maximum	38,7	11,2	7,2	5,3	2,8	3,7	4,5

Continuation to table 11

5- (OTUs 5)	SVL	HL	HW	HH	OD	EED	SED
N = 2 males							
Mean	50,600	16,450	10,400	6,000	3,250	4,250	6,500
Standard deviation	3,3941	1,2021	,5657	,4243	,0707	,0707	,1414
Minimum	48,2	15,6	10,0	5,7	3,2	4,2	6,4
Maximum	53,0	17,3	10,8	6,3	3,3	4,3	6,6
N = 2 females							
Mean	33,950	11,850	7,000	4,900	3,050	2,900	5,050
Standard deviation	7,0004	1,6263	1,4142	,8485	,4950	,4243	,7778
Minimum	29,0	10,7	6,0	4,3	2,7	2,6	4,5
Maximum	38,9	13,0	8,0	5,5	3,4	3,2	5,6
6- (OTUs 6)							
N = 2 males							
Mean	49,550	14,850	9,850	6,100	3,400	4,600	6,350
Standard deviation	9,2631	1,9092	2,1920	,7071	,1414	,1414	1,2021
Minimum	43,0	13,5	8,3	5,6	3,3	4,5	5,5
Maximum	56,1	16,2	11,4	6,6	3,5	4,7	7,2
N = 2 females							
Mean	55,050	15,150	9,100	5,750	3,500	4,600	5,700
Standard deviation	6,1518	1,4849	,9899	,0707	,4243	,2828	,8485
Minimum	50,7	14,1	8,4	5,7	3,2	4,4	5,1
Maximum	59,4	16,2	9,8	5,8	3,8	4,8	6,3

Continuation to table 11

7- (OTUs 7)	SVL	HL	HW	HH	OD	EED	SED
N = 1 female							
Mean	31,300	10,400	6,200	3,700	2,800	2,800	4,000
Minimum	31,3	10,4	6,2	3,7	2,8	2,8	4,0
Maximum	31,3	10,4	6,2	3,7	2,8	2,8	4,0
8- (OTUs 8) *H. robustus*							
N = 5 females							
Mean	38,360	10,880	6,860	4,680	2,680	3,300	4,440
Standard deviation	8,4559	2,4894	1,2178	,8258	,3962	,4690	,6229
Minimum	25,9	6,8	4,9	3,3	2,1	2,8	3,6
Maximum	48,0	12,7	7,9	5,5	3,1	3,8	5,0
9- *H. flaviviridis*							
N = 6 males							
Mean	64,333	19,017	13,467	7,983	4,133	5,750	8,217
Standard deviation	6,0991	1,5184	1,4067	1,0381	,1506	,5648	1,0304
Minimum	58,1	17,2	12,0	6,6	4,0	5,0	7,3
Maximum	73,9	20,8	15,1	9,1	4,4	6,4	9,7
N = 14 females							
Mean	60,979	17,293	12,007	7,421	4,021	4,986	7,507
Standard deviation	11,2080	2,3960	2,2609	1,3818	,4300	,6515	1,3753
Minimum	38,8	12,8	7,4	5,0	3,1	3,6	4,0
Maximum	78,6	20,4	15,4	9,0	4,6	5,8	9,2

The examination of the morphological characters for the Yemeni *Hemidactylus* taxa showed significant differences among these groups as revealed by ANOVA analysis. All meristic characters in males and females showed significant differences among groups except the character of (LL) in males (table 12).

For the morphometric characters, significant differences were detected in females except the characters of (OD & EED). However, no significant differences in morphometric characters were detected in males. These insignificant values, as well, were possibly related to the low number of male specimens (table 13). Therefore, the statistical analysis of the morphometric characters was ignored.

The Discriminant Factor Analysis (DFA) using the meristic and morphometric data extracted eight female groups of *Hemidactylus* in Yemen and six male groups, because there were no male samples assigned to the remaining groups (groups seven and eight). The separation in males and females was clear as no overlap was observed among the groups except the groups of OTU 1 and OTU 2 in both sexes. A limited overlap was detected between groups of OTU 1 & OTU 5, OTU 1 & OTU 8 and OTU 2 & OTU 8 in female, but in male, the overlap did not occur among groups except the groups of one and two (fig. 11).

Table 12: Results of ANOVA comparisons among Yemeni Hemidactylus species for meristic characters. One asterisk marks significance values below 0.05, three asterisks mark significance values below 0.001 and (n.s) marks insignificant values which was more than 0.05. The threshold value for the significance was (P < 0.05).

Females	***VS***	***DS***	***TD***	***UL***	***LL***	***In G***	***1st Sc***	***4th Sc***	***MP***
Value of significance	***	***	***	***	*	*	***	***	
Males									
Value of significance	***	***	***	***	n.s	***	***	*	***

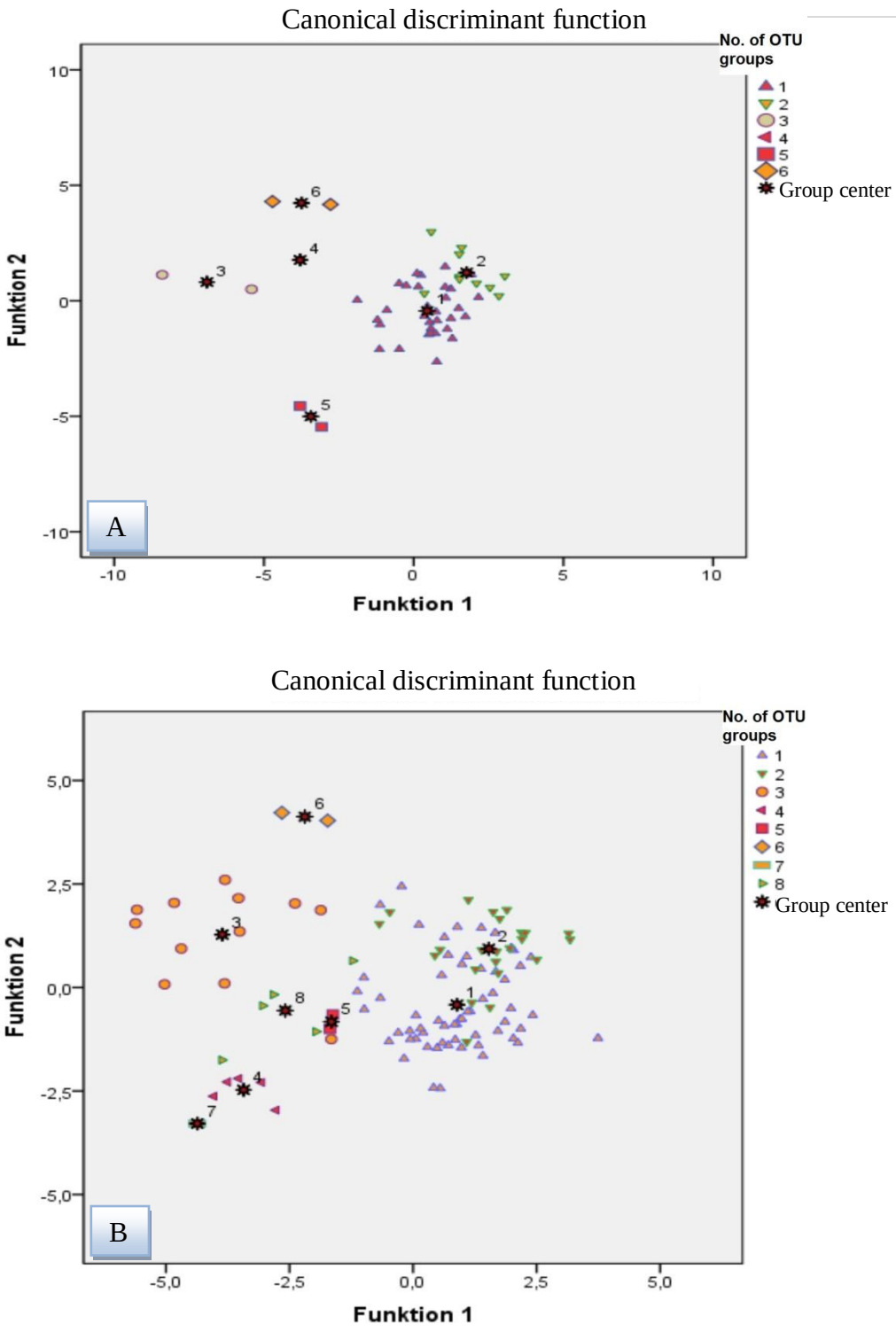


Figure 11: Classification results by DA on morphological differentiation among (A) male and (B) female *Hemidactylus* specimens from Yemen. Morphological data the same as in tables 10 & 11.

The PCA using the meristic data extracted three principle components with an eigenvalue 1 in the analysis of females and three principle components in males, these factors demonstrated 62.49 % (females) and 74.23 % (males) of the total variance.

The difference between sexes was possibly related to one of the characters found only in males. This character MP (male pores) is considerably important to produce more reliable results to distinguish species (Vences et al., 2004).

The first and second factors separated seven main groups by using scatterplots, which completely agreed with their illustration in the phylogenetic tree. The separation among males and among females was clear, although no overlap was observed among the groups except the groups of OTU 1 and OTU 2, a limited overlap between groups of OTU 1 & OTU 5, OTU 1 & OTU 8 and OTU 2 & OTU 8 in females. However in males, the overlap occurred only between groups of OTU 1 and OTU 2 (fig. 12).

In the PCA analysis of both sexes using the morphometric data from table 10 & 11 extracted three factors with an eigenvalue 1 in the analysis of males and only one factor in females. In males, these factors demonstrated 76.75 % of the total variance. However, the scatter plots based on these variables did not produce any substantial results respective for the separation of the male groups.

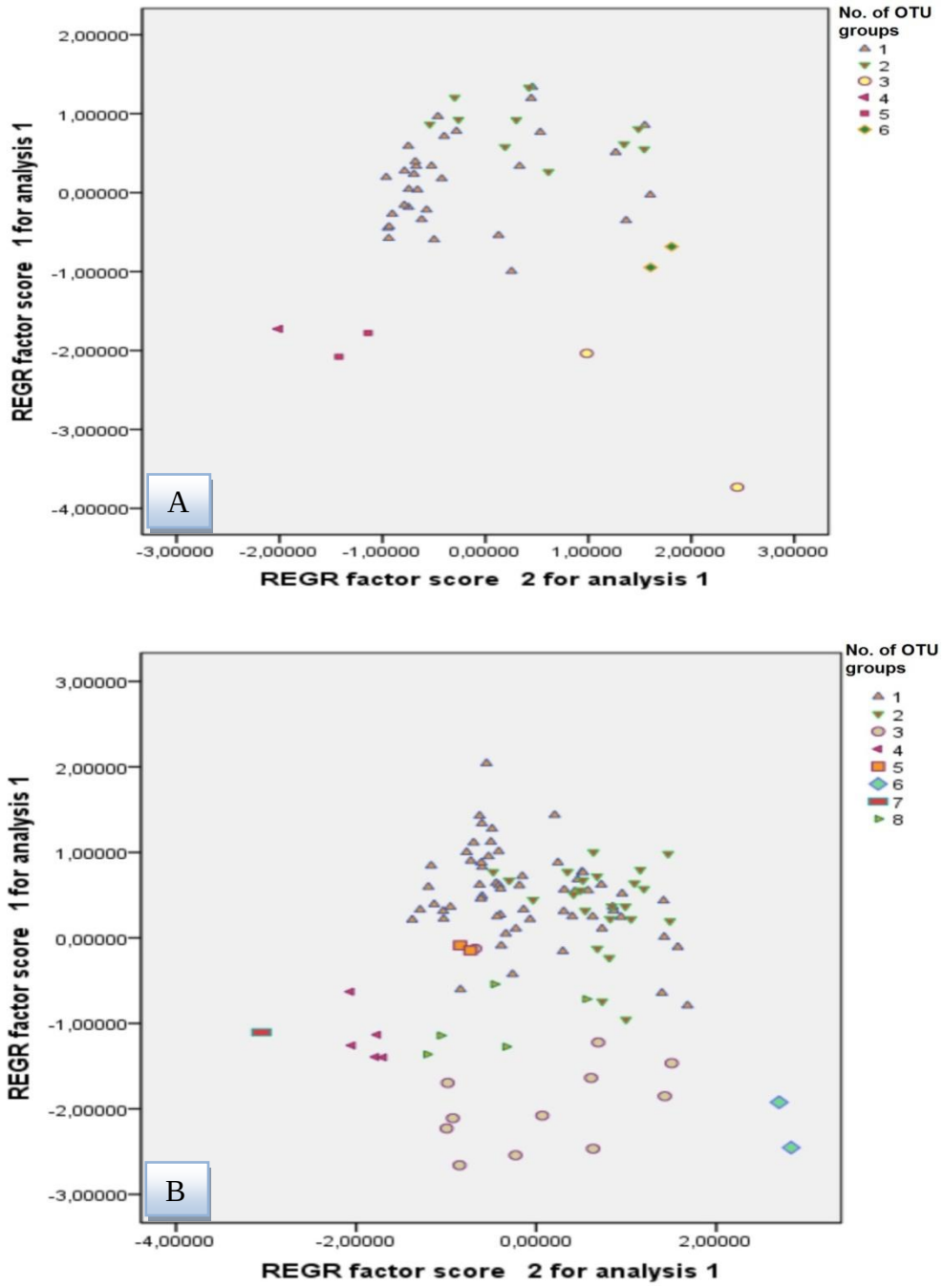


Figure 12: Morphological differentiation among *Hemidactylus* specimens from Yemen. The scatter grams show (A) male and (B) females ordered along first and second principal components of a PCA based on meristic data from table 10.

The analysis of T-test was applied among the clades which had overlapped in PCA analysis and appeared as a sister group in the phylogenetic tree of mitochondrial genes to find significant characters among these clades. To confirm the results of the morphological characters for the Yemeni *Hemidactylus* populations, the analysis of Mann-Whitney test (U-test) was yielded. This analysis revealed significant differences among groups, which had overlapped in PCA analysis and between taxa which appeared as a sister group (see fig. 7-9). The results in Mann-Whitney test (U-test) is the same as in T-test (table 13, 14). The results are shown as the following:

Four morphological characters (DS, In G., 1st Sc. and SVL) in females displayed significant differences between OTU 1 determined as *H. yerburii* ssp. and OTU 2 assigned as *H. y. yerburii* (see pp. 126 for more detail). However in males, five morphological characters (DS, 1st Sc., MP and SVL) displayed significant character between OTU 1 and OTU 2. Four meristic characters (VS, DS, TD and In G) and two morphometric characters (HH & ED) revealed in females showed significant differences between OTU 1 and OTU 3 determined as *H. yerburii ssp.* and *H. sp*.

Five meristic characters (DS, TD, In G, 1st Sc. and 4th Sc.) showed significant differences in males, in addition to one significant difference appeared among the morphometric characters. Four meristic characters (VS, DS, TD and 1st Sc.) and four morphometric characters (SVL, HH, ED and SED) revealed in females showed significant differences

between OTU 2 and OTU 3. However, three meristic characters (DS, TD, and MP) showed significant differences in males and two significant difference appeared among the morphometric characters (SVL and HH) (table 13).

For the group of *H. robustus* gecko, T-test and Mann-Whitney test were applied only on the female specimens since only one male specimen was collected throughout the study.

Three meristic characters (TD, 1st .Sc and 4th Sc.) revealed in female showed significant differences between OTU 4 and OTU 8 determined as *H. sinaitus* and *H. robustus* (table 14). However, no significant difference appeared among the morphometric characters.

Three morphological characters (VS, 1st .Sc and 4th Sc.) revealed in both sexes showed significant difference between OTU 5 and OTU 6 determined as new group species. However, no significant difference among the morphometric characters was shown.

Table 13: The results of T-test and Mann-Whitney test (U-test) comparisons among the groups of *Hemidactylus yerburii* from the mainland of Yemen by meristic and morphometric characters. One asterisk marks significance values below 0.05, two asterisks mark significance values below 0.01 and three asterisks mark significance values below 0.001 and (n.s) marks insignificant values which were more than 0.05. The threshold value for the significance was ($P < 0.05$).

	T-test						***U-test***					
	OTU 1 Vs OTU 2		OTU 1 Vs OTU 3		OTU 2 Vs OTU 3		OTU 1 Vs OTU 2		OTU 1 Vs OTU 3		OTU 2 Vs OTU 3	
Sex	F	M	F	M	F	M	F	M	F	M	F	M
VS	n.s.	n.s.	***	n.s.	***	n.s.	n.s.	n.s.	***	n.s.	***	n.s.
DS	***	*	***	***	***	***	**	*	***	**	***	*
TD	n.s.	n.s.	***	***	***	**	n.s.	n.s.	***	**	***	*
UL	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
LL	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
In G	*	n.s.	***	***	n.s.	n.s.	*	n.s.	***	*	n.s.	n.s.
1st Sc	***	**	n.s.	*	**	n.s.	***	*	n.s.	*	*	n.s.
4th Sc	n.s.	n.s.	n.s.	**	n.s.	n.s.	n.s.	n.s.	n.s.	*	n.s.	n.s.
MP		***		*		**		***		n.s.		*
SVL	**	*	n.s.	n.s.	**	*	**	*	n.s.	n.s.	**	*
Rel. LH	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Rel. HW	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

Continuation to table 13

Rel. HH	n.s.	n.s.	*	**	*	**	n.s.	n.s.	*	*	*	*
Rel. OD	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Rel. EED	n.s.	*	*	n.s.	*	n.s.	n.s.	*	**	n.s.	**	n.s.
Rel. SED	n.s.	n.s.	n.s.	n.s.	**	n.s.	n.s.	n.s.	n.s.	n.s.	*	n.s.

Table 14: The results of T-test and Mann-Whitney test (U-test) comparisons among the remaining groups of Yemeni *Hemidactylus* clades (OTU 4 Vs OTU 8) and (OTU 5 Vs OTU 6), by meristic characters in addition to one morphometric character (SVL) for both sexes. One asterisk marks significance values below 0.05, two asterisks mark significance values below 0.01 and three asterisks mark significance values below 0.001 and (n.s) marks insignificant values which were more than 0.05. The threshold value for the significance was (P < 0.05).

	T-test		***U-test***	
	OTU 5 Vs OTU 6	OTU 4 Vs OTU8	OTU 5 Vs OTU 6	OTU 4 Vs OTU 8
VS	***	n.s.	*	n.s.
DS	n.s.	n.s.	n.s.	n.s.
TD	n.s.	**	n.s.	*
UL	n.s.	n.s.	n.s.	n.s.
LL	n.s.	n.s.	n.s.	n.s.
In G	n.s.	n.s.	n.s.	n.s.
1^{st} Sc		***	*	**
4^{th} Sc		*	*	*
MP	n.s.		n.s.	
SVL	n.s.	n.s.	n.s.	n.s.

Discussion

More than twenty species concepts were proposed to explain the term ‘species’. However, several of these concepts could be summarized under the Biological Species Concept as advocated by Hennig, Ecological Species Concept, the Phylogenetic Species Concept and other species concepts (Willmann 2010).

The generally known species concept is the biological species concept suggested by Ernst Mayr. In his definition, he described a species as a group of populations whose members have the potential to interbreed in nature and produce viable, fertile offspring but do not produce viable fertile offspring with members of other such groups (Mayr 1942). However, some biologists, including proponents of the biological species concept, have argued that no species concept is universally applicable across all organisms (Carcraft 1987). The strength of the biological species concept is that it directs the attention to how speciation occurs by the evolution of reproductive isolation. However, the number of species to which this concept can be usefully applied is limited, since there is no way to evaluate the reproductive isolation of fossils. Furthermore, it does not apply to organisms that reproduce asexually all or most of the time and species on islands (real or isolated habitats on land) (Campbell et al., 2008). For these reasons and others, the additional species concepts emerged which were proposed to fulfill the research questions.

The phylogenetic species concept defines a species as the smallest group of individuals that share a common ancestor, forming one branch on the tree of life. In this analysis, biologists follow the phylogenetic history of a species by comparing its

characteristics, with those of other organisms. Any distinguished groups of individuals in this analysis are considered separate species (Campbell et al., 2008).

The dependence on one species concept in any study is insufficient to obtain accurate results since each species concept has advantages and disadvantages. However, the use of the phylogenetic species concept is more precise to determine populations that can be assigned to groups related to specific species or not. This concept is established more accurately particularly when applied along with another approach. The use of morphological analysis to study the differences among species has several advantages as it can be applied to asexual and sexual organisms, and can be useful even without information on the extent of gene flow. In this approach, most scientists can distinguish numerous species especially in the field (Campbell et al., 2008).

The distinction among the species within *Hemidactylus* using superficial features is taxonomically difficult. This is due to the considerable variation in the range of external characters such as body size, size of dorsal scales and absence of enlarged dorsal tubercles -when present, their number, size and shape and other morphological characters. This variation makes it hard to construct clear identification keys for them (Spawls 2002, Carranza and Arnold 2006). As a result of these systematic problems in *Hemidactylus*, the phylogenetic species concept, using molecular methods, often simplifies distinguishing the species as in other genera that have similar problems.

The genus *Hemidactylus* is one of the species rich genera of the family Gekkonidae. This genus is ubiquitous. Inspite of the diversification in number of species in Yemen, this genus, like other lizards, is one of the most poorly studied groups of reptiles in Yemen. In addition, the previous studies on lizards conducted in Yemen

depended only on morphological differences, which cannot detect cryptic species. Moreover, previous studies mentioned that several species occurred in the mainland of Yemen: *Hemidactylus flaviviridis, H. homoeolepis, H. lemurinus, H. persicus, H. robustus, H. sinaitus* (*shugraensis*), *H. turcicus* and *H. yerburii*. However, the records of *H. homoeolepis, H. persicus* and *H. turcicus* need confirmation to prove the occurrence of such species in the mainland. Investigating the occurrence of these species in Yemen is important to clarify the status of this genus in the mainland. Therefore, the *Hemidactylus* groups have been distinguished by constructing mitochondrial gene trees for all specimens by sorting them into groups according to their locations and similarities, then studying and comparing the morphological characters of OTU groups by using statistical tests. The differences that appeared among the OTUs arose possibly due to the diversity in climatic conditions and variations in topographic areas.

Phylogeny

The results revealed that the Yemeni geckos refer to the 'arid clade' and are consistent with the findings of Carranza and Arnold (2006) except one sequence of *Hemidactylus* sp. from Najran in Saudi Arabia assigned as *H. yerburii* in Genbank (discussed below).

As is mentioned previously, the Yemeni *Hemidactylus* species in the mainland are divided into three monophyletic groups, in addition to two monophyla of the Socotran clades. The OTUs and clades in this chapter are identical except the single sequence of OTU 7. These groups are:

Group of *Hemidactylus yerburii*

This group is found in the mainland. It forms three monophyletic clades: the first and second clades are OTU 1 and OTU 2 that are assigned to the species of *H. yerburii*, and the third clade is OTU 3. The members of the OTU 2 are distributed in the coastal plain of Yemen. However, the members of the OTU 1 are from the high mountains and mountain basins, which occur in the same area of the populations of OTU 3 (sympatric). The phylogenetic results confirm the morphological findings that the three monophyletic populations of *H. yerburii* group represent three distinct taxa. The question then emerges as to whether these three taxa should be recognized as species or subspecies.

In the case of OTU 1 and OTU 2 in all mitochondrial trees, there is a close genetic relationship as well as deep divergence between these two clades (except in PDC). This provides additional support to the suggestion that these two clades are referring to two distinct subspecies of *H. yerburii*. Moreover, the genetic distance between these two clades is relatively high (11 %) in the cytochrome *b* gene, 6 % in 12 S and 8% in the combined mitochondrial genes (table 15-17). In addition, the morphological data present several significant characters in the number of the dorsal scales, number of scansors under the 1st toe; internasal granules and male pores (table 13). In PCA, considerable variations were observed between the members of OTU 1 and OTU 2. Furthermore, the two populations of OTU 1 and OTU 2 are distributed in different areas as described above (allopatric).

Since there is no differentiation obtained in PDC nuclear gene, the suggestion for the current taxonomy status of these two clades that represent two subspecies of one species appears valid.

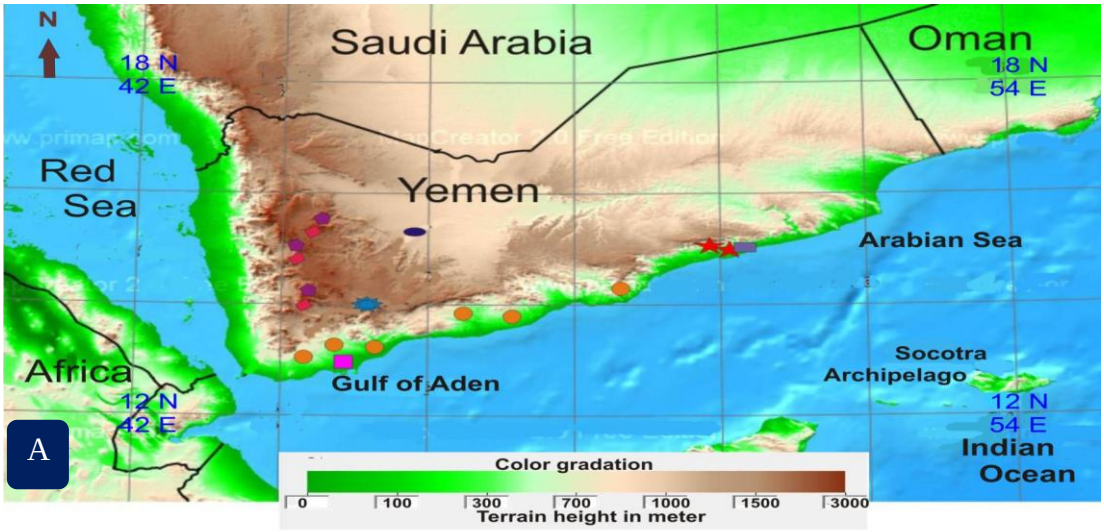
In case of the OTU 3 clade, there is a considerable genetic distance in the cytochrome *b* gene, approximately 11 – 13 % between this clade and the other clades of *H. yerburii*, 6 – 7 % in 12S and 8 – 10 % in the combined mitochondrial genes (table 15-17). The most notable result is that the tree of the PDC separates this clade with a strong bootstrap support. Furthermore, there is a deep genetic divergence between the two clades of *H. yerburii* and the members of OTU 3 in all phylogenetic trees (including PDC) confirming that the clades of this group represent distinct taxa. In addition, the morphological data present several significant characters in the number of the ventral scales, dorsal scales, tubercle dorsal scales, internasal granule and male pores and to the number of scansors under the first and fourth toe between the populations of OTU 3 and *H. y. yerburii* (OTU 2). Moreover, several significant characters appeared in the number of the ventral scales, dorsal scales, tubercle dorsal scales, internasal granule, male pores and the number of scansors under the first and fourth toe between the populations of OTU 3 and OTU 1 (table 13). In PCA, high variations were observed among the specimens of OTU 1, OTU 2 and OTU 3, which separate the populations of OTU 3 clearly than the OTU 1 and OTU 2.

It is apparent that the populations of both of the OTU 1 and OTU 3 occur in the same region (sympatric). These evidences in addition to the results of morphological tests confirm that the members of clade OUT 3 represent a new species (see pp. 137 for more detail).

According to Carranza and Arnold (2006), one sequence of *Hemidactylus* identified in Genbank as *H. yerburii* from Najran, south of Saudi Arabia has a close relationship with the group of *H. mabouia*. However, the status of this sequence is not clear, since different relationships appear with several species in different trees. For instance, in the tree of 12S gene it is clustered with the group of *Hemidactylus foudaii* and *H. citernii*, whereas in the cytochrome *b* tree it is aligned with the group of OTU 5, OTU 6 and OTU 7 with weak bootstrap support in both analysis of Bayesian and ML.

This study confirms that this sequence is not related to the species of *H. yerburii* since it does not align with any known clades, however, it appears a different relationship with different group in different gene trees. Furthermore, as it is mentioned before, the members of OTU 2 are appropriate to the description of holotype specimens, which was mentioned by Anderson (1895). In addition, the specimens were collected at the type locality. Furthermore, they fit to the samples identified as *H. yerburii* from (MTKD) museum and (ZFMK) museum. The ambiguous status of this sequence may indicate that it belongs to another species or represents a new species; therefore, it needs further morphological studies to clarify its position. Thus, the classification of this sequence as *H. yerburii* is misidentified probably due to cryptic features.

The clade of *H. yerburii* group is closely related to the endemic Socotran species of *H. pumilio* and the Persian gecko of *H. persicus* from Oman in the tree of mitochondrial genes. This study confirms the finding of Carranza and Arnold (2006) that *H. pumilio* is a sister to *H. dracaenacolus* and *H. granti* with very low bootstrap support and they have a close relationship with *H. persicus*, but in present study it is more closer to the Persian geckos with high bootstrap support.


N
Saudi Arabia
Oman
18 N
42 E
18 N
54 E
Red
Sea
Yemen
Arabian Sea
Africa
Gulf of Aden
Socotra
Archipelago
12 N
42 E
12 N
54 E
Indian
Ocean
A
Color gradation
0
100
300
700
1000
1500
3000
Terrain height in meter

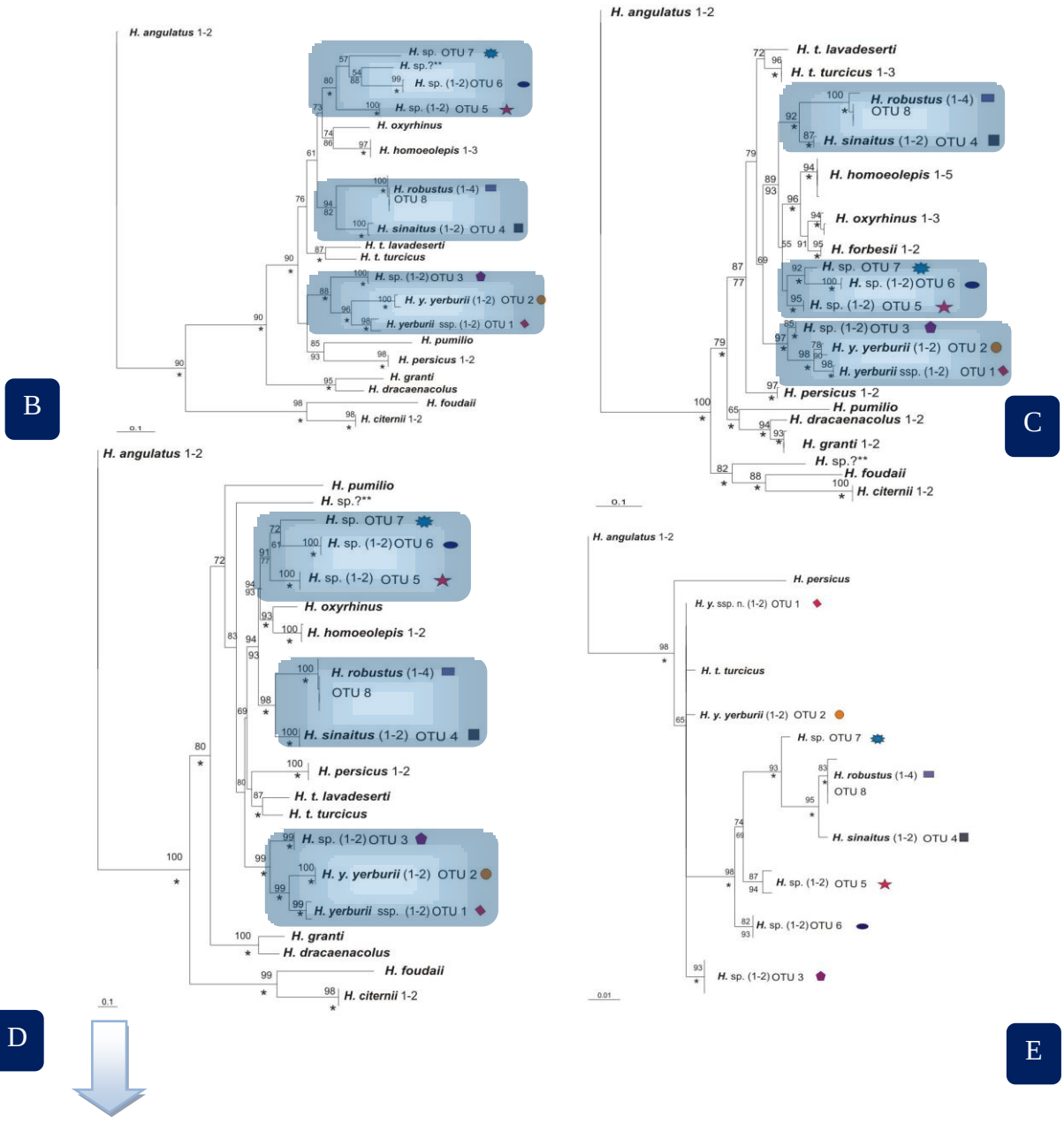

B
H. angulatus 1-2
H. sp. OTU 7
H. sp.?**
H. sp. (1-2)OTU 6
H. sp. (1-2) OTU 5
H. oxyrhinus
H. homoeolepis 1-3
H. robustus (1-4) OTU 8
H. sinaitus (1-2) OTU 4
H. t. lavadeserti
H. t. turcicus
H. sp. (1-2)OTU 3
H. y. yerburii (1-2) OTU 2
H. yerburii ssp. (1-2) OTU 1
H. pumilio
H. persicus 1-2
H. granti
H. dracaenacolus
H. foudaii
H. citernii 1-2
C
H. angulatus 1-2
H. t. lavadeserti
H. t. turcicus 1-3
H. robustus (1-4) OTU 8
H. sinaitus (1-2) OTU 4
H. homoeolepis 1-5
H. oxyrhinus 1-3
H. forbesii 1-2
H. sp. OTU 7
H. sp. (1-2)OTU 6
H. sp. (1-2) OTU 5
H. sp. (1-2)OTU 3
H. y. yerburii (1-2) OTU 2
H. yerburii ssp. (1-2) OTU 1
H. persicus 1-2
H. pumilio
H. dracaenacolus 1-2
H. granti 1-2
H. sp.?**
H. foudaii
H. citernii 1-2
D
H. angulatus 1-2
H. pumilio
H. sp.?**
H. sp. OTU 7
H. sp. (1-2)OTU 6
H. sp. (1-2) OTU 5
H. oxyrhinus
H. homoeolepis 1-2
H. robustus (1-4) OTU 8
H. sinaitus (1-2) OTU 4
H. persicus 1-2
H. t. lavadeserti
H. t. turcicus
H. sp. (1-2)OTU 3
H. y. yerburii (1-2) OTU 2
H. yerburii ssp. (1-2)OTU 1
H. granti
H. dracaenacolus
H. foudaii
H. citernii 1-2
E
H. angulatus 1-2
H. persicus
H. y. ssp. n. (1-2) OTU 1
H. t. turcicus
H. y. yerburii (1-2) OTU 2
H. sp. OTU 7
H. robustus (1-4) OTU 8
H. sinaitus (1-2) OTU 4
H. sp. (1-2) OTU 5
H. sp. (1-2)OTU 6
H. sp. (1-2)OTU 3

Figure 13: (A) Distribution of mitochondorial lineages of *Hemidactylus* in the mainland of Yemen. (B) ML trees for: (B) cyt *b*. gene (C) 12S gene (D) a combination of the cytochrome *b* and 12S rRNA mtDNA sequences obtained with PHYML (E) PDC nuclear gene. Numbers by the nodes indicate: for ML bootstrap values (> 50%) are given above the nodes and Bayesian probabilities are given below the nodes. An asterisk indicates a posterior probability of ≥ 0.95.

****: A sequence of *Hemidactylus* from Najran, Saudi Arabia, identified in Genbank as *H. yerburii*.**

***** The numbers between brackets refer to the samples in table 8 and 9.**

Table 15: Uncorrected genetic distances for the Cytochrome b gene fragment used in this study.

	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*
OTU 1	-							
OTU 2	0.11	-						
OTU 3	0.11	0.13	-					
OTU 4	0.16	0.17	0.16	-				
OTU 5	0.15	0.16	0.15	0.13	-			
OTU 6	0.16	0.17	0.16	0.15	0.12	-		
OTU 7	0.17	0.19	0.17	0.16	0.13	0.14	-	
OTU 8	0.17	0.17	0.17	0.12	0.14	0.17	0.15	-
Out group	0.22	0.22	0.22	0.20	0.20	0.21	0.21	0.20

Table 16: Uncorrected genetic distances for the 12S gene fragment used in this study.

	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*
OTU 1	-							
OTU 2	0.06	-						
OTU 3	0.07	0.05	-					
OTU 4	0.12	0.12	0.13	-				
OTU 5	0.11	0.11	0.12	0.09	-			
OTU 6	0.14	0.13	0.14	0.13	0.09	-		
OTU 7	0.13	0.11	0.12	0.10	0.07	0.08	-	
OTU 8	0.15	0.15	0.14	0.10	0.11	0.12	0.12	-
Out Group	0.21	0.21	0.20	0.22	0.22	0.22	0.22	0.21

Table 17: Uncorrected genetic distances for the combined gene fragments used in this study.

	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*
OTU 1	-							
OTU 2	0.08	-						
OTU 3	0.09	0.10	-					
OTU 4	0.13	0.14	0.13	-				
OTU 5	0.12	0.13	0.12	0.10	-			
OTU 6	0.14	0.14	0.13	0.12	0.10	-		
OTU 7	0.14	0.15	0.13	0.13	0.10	0.11	-	
OTU 8	0.14	0.14	0.14	0.10	0.12	0.14	0.13	-
Out Group	0.18	0.19	0.18	0.17	0.18	0.18	0.19	0.18

Table 18: Uncorrected genetic distances for the PDC gene fragment used in this study.

	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*
OTU 1	-							
OTU 2	0.00	-						
OTU 3	0.01	0.01	-					
OTU 4	0.04	0.04	0.04	-				
OTU 5	0.02	0.02	0.03	0.02	-			
OTU 6	0.02	0.02	0.02	0.03	0.02	-		
OTU 7	0.03	0.03	0.03	0.01	0.02	0.02	-	
OTU 8	0.04	0.04	0.04	0.01	0.02	0.03	0.01	-
Out Group	0.02	0.02	0.03	0.06	0.05	0.04	0.05	0.06

Group of *H. robustus*

This group is found in the mainland. It forms two monophyletic clades: the first is *H. robustus* (OTU 8) recorded in the southeast of the coastal plain, and the second clade is *H. sinaitus* (OTU 4) recorded in the southern coastal plain of Yemen (fig. 13).

The members of *H. robustus* were identical to other samples from Abu Dhabi in UAE and from Safaga in Egypt sequenced by Carranza and Arnold (2006) and form a monophyletic group. This species is more closely related to *H. sinaitus* and other Socotran and Arabian species than to *H. turcicus*.

Carranza and Arnold (2006) and Bauer et al., (2006a) mentioned that *H. robustus* was distinct from *H. turcicus*, and both species were not close relatives. The present study confirms these results. The tree of PDC separates this clade with a high bootstrap support in both analysis of Bayesian and ML.

The genetic distances between the two major lineages of this group are 12 % in the cytochrome *b*, 10 % in 12S and 10 % in the combined mitochondrial genes (table 15, 16, 17), and approximately 1 % in the nuclear gene (table 18). The morphological data present some significant differences between these two clades in the number of the dorsal tubercle scales, number of scansors under the first and fourth toe (table 14).

The clade of *H. robustus* group is closely related to the second group of Socotran archipelago species of *H. homoeolepis* and *H. oxyrhinus* in the cytochrome *b* tree in addition to *H. forbesii* in the tree of 12S, and to the group of undescribed *Hemidactylus* species.

Group of undescribed *Hemidactylus* species

This group forms two monophyletic clades in addition to one distinct sequence. The members of this group are not related to any known species. The first clade is OTU 5 from the coastal plain in southeastern Yemen. The second clade is OTU 6 from the desert. The single sequence of OTU 7 is from the high mountains (fig. 13).

The phylogenetic results confirm the morphological findings that the monophyletic populations of this new group represent three distinct taxa. The question arises again as to whether these three taxa should be recognized as species or subspecies.

All mitochondrial trees in addition to the nuclear tree separate the population of these units in distinct clades with high bootstrap support. The deep divergence among these two clades of OTU 5 and OTU 6 and the one single sequence of OTU 7 provide additional support to the suggestion that these three units are referring to three distinct species. Moreover, the genetic distance between these clades is high (12 % - 13 %) in the cytochrome *b* gene, 7 – 9 % in 12S and 10 - 11 % in the combined mitochondrial genes (table 15, 16, 17). Furthermore, the genetic distance in the nuclear gene is approximately 2 % (table. 18). In addition, the PCA illustrated a clear separation among the species in this group. Moreover, the comparison between morphological data of OTU 5 and OTU 6 present several significant differences within these units in the number of ventral scales, dorsal tubercle scales and the number of scansors under the first and fourth toe. Thus, the morphological characters support the results of the phylogenetic trees. Such evidences confirm that the three populations of these geckos (OTU 5, OTU 6 and OTU 7) belong to three distinct species.

These three populations are distributed in different areas as described above (allopatric). Since there is differentiation obtained in PDC, the suggestion for the current taxonomy status that these units contain three new species is valid.

The new species group is closely related to the second group of Arabian and Socotran archipelago species, in addition to *H. oxyrhinus*, which is found in Abd el Kuri island in the cytochrome *b* tree, and to *H. forbesii* in the tree of 12S.

Socotra has been colonized by *Hemidactylus* four times, two early colonizations leading to endemic groups: *H. homoeolepis, H. forbesii* and *H. oxyrhinus, H. pumilio, H. dracaenacolus* and *H. granti*; two later invasions: *H. turcicus* and *H. flaviviridis*).

Recorded taxa and undescribed taxa in the mainland of Yemen

In this section, all species that have been mentioned in the literature are listed and discussed (see the introduction pp. 32-34). In addition to the known species that were found during this study and undescribed species are presented.

1. *Hemidactylus flaviviridis* Rüppell, 1835

Hemidactylus flaviviridis, Rösler and Wranik 1998

Type locality: Abyssinia

H. flaviviridis is distinguished from all other species of *Hemidactylus* in Yemen by the size, which is medium to large, and not having dorsal tubercles. This species is distributed along the coast of the Red Sea in Africa from Egypt to Eritrea and northern Somalia, and from the periphery of Arabian coasts to Iraq, southern Iran, Afghanistan, Pakistan and northern India (Anderson 1898, Arnold 1986, Baha El Din 2005). According to Lanza (1983) and Baha El Din (2005) the populations found in Somalia and Egypt have been introduced. This confirms the idea that the origin of this species was from central India and has spread towards the west by trade routes (Anderson 1999).

This species is clustered within the tropical Asian clade (Carranza and Arnold 2006). In Yemen, *H. flaviviridis* is known to exist on the coastal plain where it favors old buildings. It is recorded in Aden, Taiz, Al-Hudaidah, Abian, Lahj and Hadhramout Governorates, in addition to Socotra Island. Throughout this study, several samples were collected from Aden, Radfan, Zindjebar in Abian, Mahfid, Mukalla, Tebala and Ash-Shihr in the coastal plain of the south of Yemen.

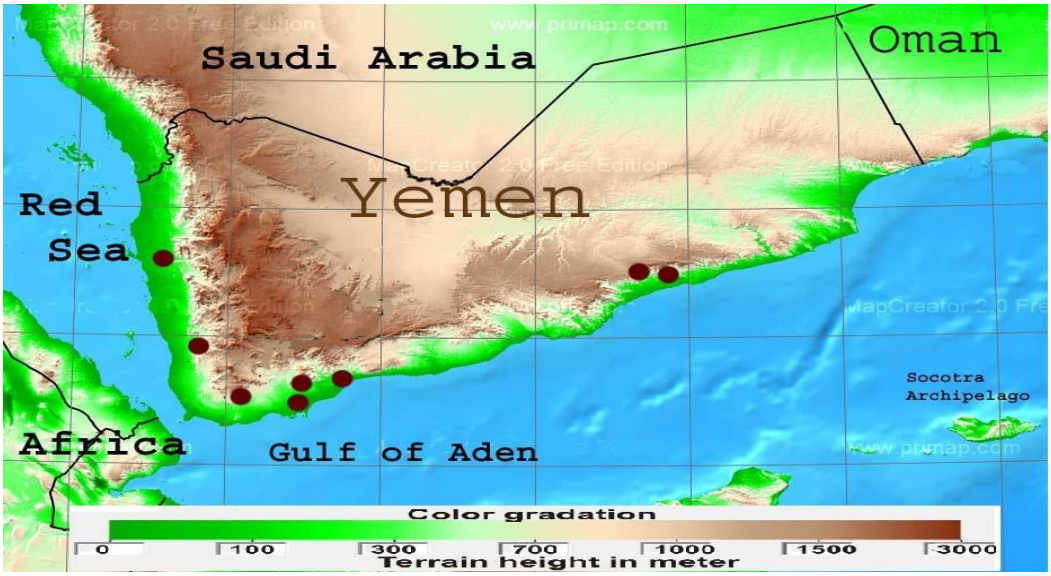


Figure 14: Distribution of *Hemidactylus flaviviridis* in the mainland of Yemen.

Figure 15: dorsal view of *Hemidactylus flaviviridis,* male, from Al-Mukalla (NHM-BS N42051).

2. *Hemidactylus homoeolepis* Blanford, 1881

Hemidactylus homoeolepis, Arnold 1977

Type locality: Socotra.

This species is recorded in the Socotra Archipelago in the islands of Socotra, Abd al-Kuri and Samha, and the periphery of southern Arabia from Al-Qunfudhah in the Red Sea to Dhofar, the Kuria Muria Islands and Masirah Island in Oman (Arnold 1980, Schätti and Gasperetti 1994, Schätti and Desvoignes 1999, Wranik 2003, Joger 2000).

The occurrence of this gecko in the mainland of Yemen is based on one specimen from Shugra (Arnold 1980, Schätti and Desvoignes 1999).

Throughout this study, no samples referring to this species were found in the mainland. Tissues from this species collected from Socotra and identified by Prof. Dr. Ulrich Joger, were extracted, sequenced, examined and clustered within this study with the sequences of *H. homoeolepis* clade from Socotra that is identified from the Genbank by Carranza and Arnold (2006) (fig. 13, table 8, 9).

H. homoeolepis was recorded only once from Shugra in the southern Yemen since 1977 (Schätti and Desvoignes 1999). Since then, no other samples were recorded. Furthermore, no specimens referring to this species were found during this study, however, several specimens were collected from the neighboring area of Shugra assigned to *H. y. yerburii*. Moreover, two specimens of *H. y. yerburii* were observed in Shugra at night on the wall of an old building.

Actually, the dependence on a single specimen to prove the record of *H. homoeolepis* in Shugra is doubtful since it probably occurred due to accidental introduction as Shugra is a harbor, and fishermen travel from Socotra to Shugra and vice versa.

The possibility exists that this single specimen was transported through one of these routes. Noticeably, the local people dislike these geckos, in addition to that the number of specimens transferred might have been considerably low, accordingly, these specimens could have been abolished soon after their entrance.

This scenario gives a reasonable explanation for why only one specimen referring to this species was recorded once in that area. Furthermore, the character of these collected specimens from Shugra resembles the specimens described from Socotra.

For these reasons, in addition to the fact that there are no further records of this species in the Shugra area since 1977, nor from neighboring area, the prediction that this species does not exist in Shugra is sound. Further studies to investigate the area located in the east part of Yemen are required to clarify the existence of *H. homoeolepis* in the mainland of Yemen.

3. *Hemidactylus lemurinus* Arnold, 1980

Hemidactylus lemurinus, Schätti and Desvoignes 1999

Type locality: Ayun, Dhofar.

This species is very similar to *H. flaviviridis*, but the snout is shorter and broader; limbs more slender; tail thinner, without tubercles; preanal pores present instead of femoral pores.

H. lemurinus is recorded in Dhofar in specific habitat, and it shows a very discontinuous distribution (Arnold 1980). Schätti and Desvoignes (1999) recorded four specimens from Wadi Hajir in Hadhramout and one specimen from Sayhut in Al-Mahra governorate.

Throughout this study, no samples referring to this species were found.

Further studies on the area near Oman's borders may register new distribution records.

4. *Hemidactylus persicus* Anderson, 1872

Hemidactylus persicus, Wermuth 1965

Type locality: Shiraz, Iran (Smith 1935)

This species is characterized by a large body size and adhesive pads on digits strongly expanded, much broader than the toe (Arnold 1986).

H. persicus is distributed from northeastern Arabia, Iraq south to Bahrain, northern Oman, southern Iran to Pakistan and India (Arnold 1986).

During the recent study, no specimens referring to the Persian Gecko were found. Moreover, no sequenced tissues collected during field work were clustered with the sequences of *H. persicus* clade identified in the Genbank by Carranza and Arnold (2006) neither were they found to be related, excluding the clade of *H. pumilio* and *H. persicus* in the cyt *b* tree (fig. 13).

The report of endangered animals in Yemen (2005) included this species in the list of endangered animals considering it could potentially occur in Yemen. However, this is inaccurate information since the area of *Hemidactylus persicus* is recorded in northeastern Arabia and in Al-Jabal Al-Akhdhar (Green Mountain) in Oman. This area is mountainous and the distance from Al-Jabal Al-Akhdhar to the borders of Yemen is too far. Furthermore, there is a natural barrier between this area and the borders of Yemen. These conditions decrease the probability of finding the populations of this species in the mainland of Yemen.

5. *Hemidactylus robustus* Heyden, 1827

Hemidactylus parkeri, Lanza 1978.

Hemidactylus turcicus parkeri, Arnold 1980

Hemidactylus robustus, Lanza 1990.

Type locality: Abyssinia

The identity of the *Hemidactylus* populations inhabiting the Arabian coast has been under debate, because many authors assigned these geckos as a synonym or subspecies to *H. turcicus* due to confusion over taxon boundaries and the lack of a thorough revision of the *H. turcicus* group resulting in the continued explicit or implicit synonymization of *H. turcicus* and *H. robustus* (Bauer et al., 2006a).

H. robustus has priority over *H. karachiensis* Murray, 1884 and *H. parkeri* Loveridge, 1936, all of which had been used for certain *H. turcicus*-like geckos (Salvador 1981, Bauer et al., 2006a). Lanza (1990) and Moravec and Böhme (1997) reviewed the nomenclatural history of the group of *H. turcicus*, and they consider *H. robustus* as an entire species. Baha El Din (2005, 2006) indicated that *H. turcicus* and *H. robustus* are present in sympatry along the Egyptian Red Sea coast and used this as a confirmation for the recognition of *H. robustus* as a separate species. Recently there is a consensus that *H. robustus* is the oldest available name appropriate to those populations (Baha El Din 2005, 2006; Carranza and Arnold 2006, Bauer et al., 2006a). This study considers that the populations which occur in the mainland of Yemen are fit to refer to the nomenclature of Salvador (1981), Lanza (1990) and Moravec and Böhme (1997) since two widely sympatric populations

referable to both *H. robustus* and *H. turcicus* occure along Egyptian red Sea coast (Baha el Din 2005, 2006). In addition, recent studies confirm that *H. robustus* and *H. turcicus* are separate species (fig. 13) (Carranza and Arnold 2006, and Bauer et al., 2006a).

Furthermore, a large difference between the gene sequences of both species of *H. turcicus* and *H. robustus* was found through this study, and both appear in different positions in the phylogenetic tree. This result supports that *H. robustus* is a valid species and confirms the finding of Carranza and Arnold (2006) (see, *H. turcicus*, below for further details).

The species *H. robustus* extends from the east African coast to southern Egypt, Arabian coasts, east to Iran and Pakistan (Baha el Din 2005, 2006; Bauer et al., 2006a). In this study, it was collected from different localities in Ash-Shihr, from Wadi Sam'uon and near buildings in Ash-Shihr city.

Description of *H. robustus* (OTU 8) collected throughout this study

The description of OTU 8 specimens fit to the diagnosis of the species *H. robustus*. Moreover, the sequences of these specimens are identical to each other and to specimens sequenced by Carranza and Arnold (2006); in addition, there is no genetic divergence between these populations in the mitochondrial genes neither the nuclear gene.

Material: NHM-BS N41917 - NHM-BS N41920, NHM-BS N42044 from Shihr, Hadhramout.

Description:

H. robustus is a small to medium-sized depressed gecko, with maximum-recorded SVL of 48 mm. Head moderately high. Nostril bordered by rostral, three nasals and sometimes the first upper labials in contact with upper nasals. 8 - 9 upper labials; 7 - 8 lower labials. Two pairs of post-mentals present. Dorsal scales granular and smooth, 61 – 75 dorsal scales across mid-body; dorsal tubercles small, weakly keeled, arranged in 14 – 16 longitudinal rows. 36 - 39 ventral scales across mid-abdomen. Limbs are somewhat short and thick. Digital pads moderately expanded. 9 – 10 lamellae under fourth toe; 6 – 7 lamellae under first toe. Tail almost smooth dorsally, with a few small indistinct tubercles; subcaudals weakly expanded along the midline.

Dorsal coloration in some specimens are pinkish brown or yellowish pale brown, translucent. Pattern sometimes made of a series of dark brown spots, arranged transversely along mid-dorsum, but often pattern is indistinct. Pair of dark brown lines on each side of the head extending from the nasals until occipital side. Tail with

several irregular dark bands or indistinct. This species was found on buildings and from rocky structures near sandy area.

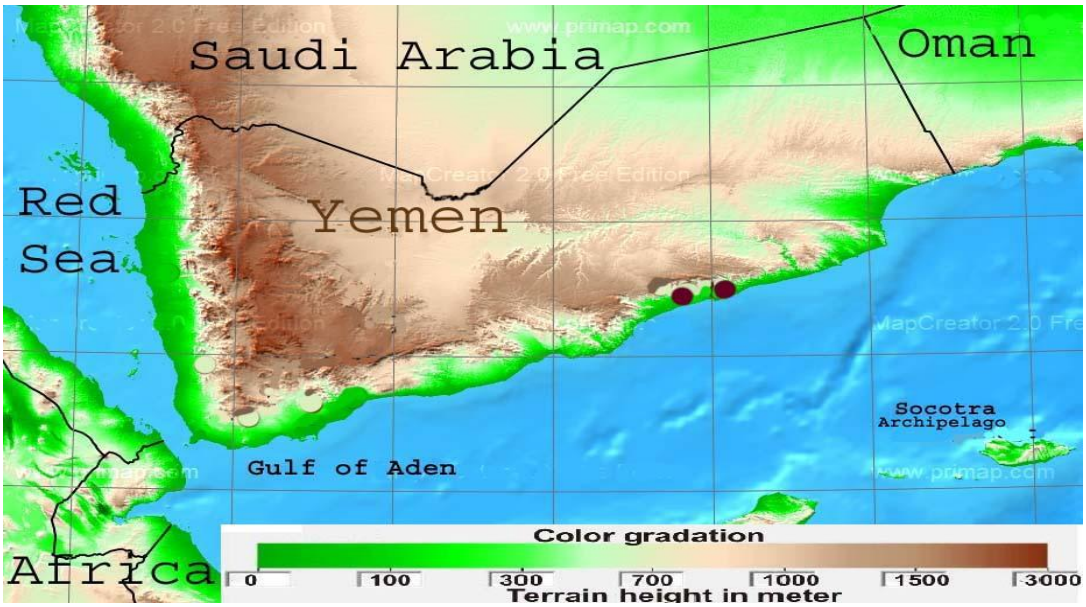


Figure 16: Distribution of *H. robustus* in the mainland of Yemen.

Figure 17: dorsal view of *H. robustus*, female, from Ash-Shihr (NHM-BS N41919).

6. *Hemidactylus sinaitus* Boulenger, 1885

Hemidactylus sinaitus, Anderson 1895 (from Sheikh Othman).

Hemidactylus shugraensis Haas and Battersby, 1959 – Type locality: Shugra (Abian)

Hemidactylus sinaitus, Arnold 1977, 1986

Type locality: Mount Sinai.

The type locality of this species reported to be 'Mount Sinai' is erroneous, it is most likely to be from the western shores of the southern Red Sea (Arnold 1977, 1986; Baha El Din 2005, 2006).

H. sinaitus is recorded from the coastal regions of Sudan, Eritrea, northern Somalia as well as from the vicinity of Aden and Shugra (Schätti and Desvoignes 1999). Haas and Battersby (1959) referred the samples collected from Shugra, Abian to the new species of *H. shugraensis*. The species *H. shugraensis* is a synonym to *H. sinaitus* (Arnold 1986, Schätti and Desvoignes 1999, Baha El Din 2006) Popov noted that this gecko appears to be common, at least locally (Haas and Battersby 1959, Schätti and Desvoignes 1999).

Throughout the study, some specimens were collected during the day on *Azadirachta indica* tree and other was near it on the ground hidden under the leaves. In Yemen, this gecko is recorded from Aden, Sheikh Othman in vicinity of Aden, Lahj and Shugra in the southern Yemen (Anderson 1895, 1901; Arnold 1986). In this study, four samples were collected from Sheikh Othman and from the same locality which Anderson (1895) described. The occurrence of this species in Aden is probably due to accidental introduction (Schätti and Gasperetti 1994).

Description of *H. sinaitus* (OTU 4) collected within this study

The other members in this monophyletic group refer to the species of *H. sinaitus* since their morphological characters correspond to the description of the species, and the locality of the present collected samples is from the same area where Anderson (1895) described *H. sinaitus* from the Yerbury collection.

Materials: NHM-BS N41902 - NHM-BS N41903 from Lahj, NHM-BS N41905 - NHM-BS N41907 from Sheikh Othman, Aden.

Description:

H. sinaitus (based on six specimens) is a small to medium-sized depressed gecko, with maximum-recorded SVL of 38.7 mm. Head moderately high. Nostril bordered by rostral, three nasals and the first upper labials in contact with upper nasals by a fine point. 8 - 9 upper labials; 7 – 8 lower labials. Two pairs of post-mentals are present, mostly expanded to the end margin of the second lower labial. Dorsal scales granular and smooth, 68 – 77 dorsal scales across mid-body; dorsal tubercles small, weakly keeled or smooth, arranged in 14 – 15 longitudinal rows. 32 – 38 ventral scales across mid-abdomen, imbricate and larger than dorsal. Limbs are somewhat short and thick. Digits are narrow and short. 9 – 10 lamellae under fourth toe; five lamellae under first toe. Male with seven pre-anal pores (in one sample). Tail almost smooth dorsally, with a few small indistinct tubercles.

The color of the dorsal side is pale brown, sometimes brownish gray, pattern made of a series of indistinct brown spots. Pair of brown lines on each side of the head extending from the nasals until occipital side. Tail with several irregular brown bands or indistinct brown spots.

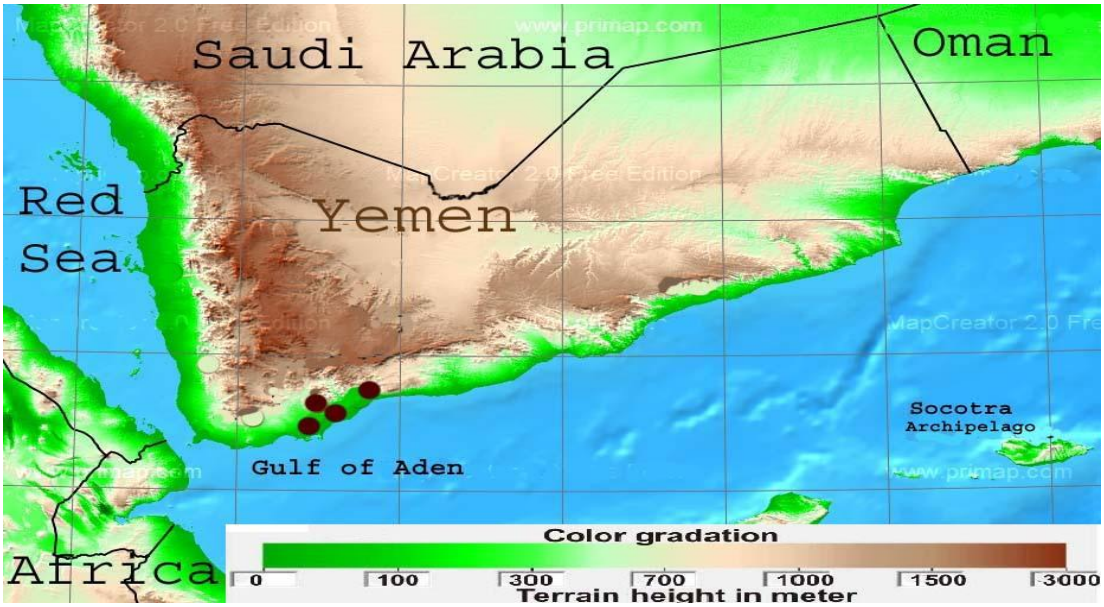


Figure 18: Distribution of *H. sinaitus* in the mainland of Yemen.

Figure 19: dorsal view of *H. sinaitus*, male, from Sheikh Othman (NHM-BS N41904).

7. *Hemidactylus turcicus* (Linnaeus, 1758)

Hemidactylus turcicus, Schmidt 1953

Type locality: Oriente (restricted to Anatolia, Schmidt 1953).

The Turkish house gecko is widespread and distributed in northern Africa and the European Mediterranean region to Pakistan, islands of the Red Sea and Arabian littoral and southern Iran. *Hemidactylus turcicus* has been widely introduced into many parts of the world. Some of the published records need further verification to establish that they do not refer to *H. robustus.* In Yemen, this species is recorded previously in Sana'a, Al-Hudaidah and south of Yemen as well as in Socotra Island.

Many workers prefer to use the specific name *H. turcicus* in its broad sense until the problem is resolved (Moravec and Böhme 1997). Most Arabian populations seem to be fit to a single taxon which is largely confined to the coastal lowlands, however, the populations that occur in high altitude are probably distinct. Therefore, the existence of more than one species belonging to these population is more valid (Arnold 1986).

As it is mentioned above (see *H. robustus*), the status and appropriateness of the *Hemidactylus* populations inhabiting the Arabian region have been under debate. Many authors impute these geckos to *H. turcicus* for simplicity (Baha El Din 2005). Lanza (1978) referred these animals to *H. parkeri*. Arnold (1980) and Fritz and Schütte (1987) discussed the systematic problems within Arabian populations and used the trinomial *H. t. parkeri*. Salvador (1981) mentioned that the suitable name for Arabian populations is *H. robustus,* and has priority over other names. Arnold (1986) and Schätti & Gasperetti (1994) chose to retain these animals under a wider

concept of the specific name *H. turcicus*. Lanza (1990), Moravec and Böhme (1997) Baha El Din (2005, 2006), Carranza and Arnold (2006) and Bauer et al., (2006a) adopted the name *H. robustus* and considered it as a separate species.

No specimens of *H. turcicus* were collected from the mainland. However, tissues referring to this species collected from Socotra and identified by Prof. Dr. Ulrich Joger were extracted, sequenced and examined within this study (fig. 8, table 8, 9). These samples are clustered in the tree of 12S gene with the sequences of *H. turcicus* clade identified in the GenBank by Carranza and Arnold (2006). Wranik (2003) referring the population of *H. turcicus* to the species *H. robustus*, however, in reality, these populations are referring to *H. turcicus* for the reasons that mentioned above.

The previous records of this species in the mainland could be referring to *H. robustus*, *H. y. yerburii*, *H. yerburii* ssp. or the undescribed species *Hemidactylus* sp. 'jumailiae'. Thus, the populations that belong to the high altitude could be referring to the undescribed species (*Hemidactylus* sp. 'jumailiae') or the subspecies (*H. yerburii* ssp.), whereas the populations that exist in the lowlands might be referring to the species *H. robustus* or *H. y. yerburii*.

8. *Hemidactylus yerburii yerburii* Anderson, 1895

Hemidactylus yerburii Anderson, 1895:640

Hemidactylus yerburii yerburii, Schätti and Gasperetti 1994

Type locality: Lahj

Hemidactylus yerburii is distributed from south-west Saudi Arabia through northern Yemen to southern Yemen and eastwards towards Dhofar (Oman) and in northern Somalia.

This species is characterized by an extremely high variation among its populations (Arnold 1986). This variation is also found in this study. Some specimens are large and robust with big prominent dorsal tubercles; the expanded subcaudals extend forwards almost to the tail base; general coloring can change to dark grayish brown and the tail has numerous dark transverse bands. However, some geckos are quite different being much smaller and more slender with smaller, less raised tubercles and the row of expanded subcaudal plates ending at the tail base; the coloring is pale with the tubercles often bearing opaque white pigments. The tail has fewer, broader transverse bands and is very contrastingly patterned in juveniles. At first view, the differences between these specimens are so marked that it is tempting to regard them as belonging to separate species. On the other hand, several geckos are intermediate, falling between the other samples in size, build, tubercles size and coloring.

Since there are several variations between Arabian and Somali animals, Lanza (1978) has named Somali material as a separate subspecies, *H. y. pauciporosus*. In addition, this study recognizes the differentiation in morphological and genetical characters between the samples collected from high altitude and those from lower

altitude in the mainland. It is possible that the differentiation found in these geckos in Yemen is probably related at least indirectly to climatic factors.

The members of OTU 2 are appropriate to the description of holotype specimens which was mentioned by Anderson (1895). In addition, the specimens were collected at the type locality. Furthermore, they fit to the samples identified as *H. yerburii* from (MTKD) museum 'Museum für Tierkunde Dresden' as well as samples from the (ZFMK) museum 'Alexander Koenig for Zoological Research, Bonn'.

Description of *H. yerburii yerburii* (OTU 2) collected within this study

Materials: NHM-BS N41856 - NHM-BS N41859, NHM-BS N41861 - NHM-BS N41866, NHM-BS N41868 - NHM-BS N41870, NHM-BS N41888 from Tour Albaha, NHM-BS N41860 from Lahj, NHM-BS N41871 - NHM-BS N41872 from Radfan, NHM-BS N41873 from Shihr, NHM-BS N41874 - NHM-BS N41875 from Aryab, NHM-BS N41876 - NHM-BS N41886 from Lowder, Abian; NHM-BS N41887 from Aden.

Description of specimens:

Body is more slender than depressed. The maximum recorded SVL is approximately 64 mm. Head sparsely covered with enlarged convex granules, the largest granules are between the eye and nostril. Nostril formed by the rostral, labial, and three nasals. Body covered with minute flat rounded granules with numerous large strongly trihedral tubercles intermixed and arranged in 14 -16 longitudinal rows. Ventral scales cycloid and imbricate, larger than dorsals. Limbs and digits are well developed, 10 – 11 lamellae under fourth toe; 6 – 8 lamellae under first toe. Males with 10 – 17 pre-anal pores. Tail is depressed, tubercles almost with strong distinct keels; arranged in 6 rows; subcaudals uniform.

General color is brownish gray, with dusky band before and behind the eye, sometimes with feeble dusky markings on the head, neck and shoulders; on dorsal side, pattern of a series of indistinct or distinct dark spots are found; faint or distinct indications of dark bands on the middle of the tail towards the tip. Ventral side is white, minutely spotted with livid on the sides of the belly.

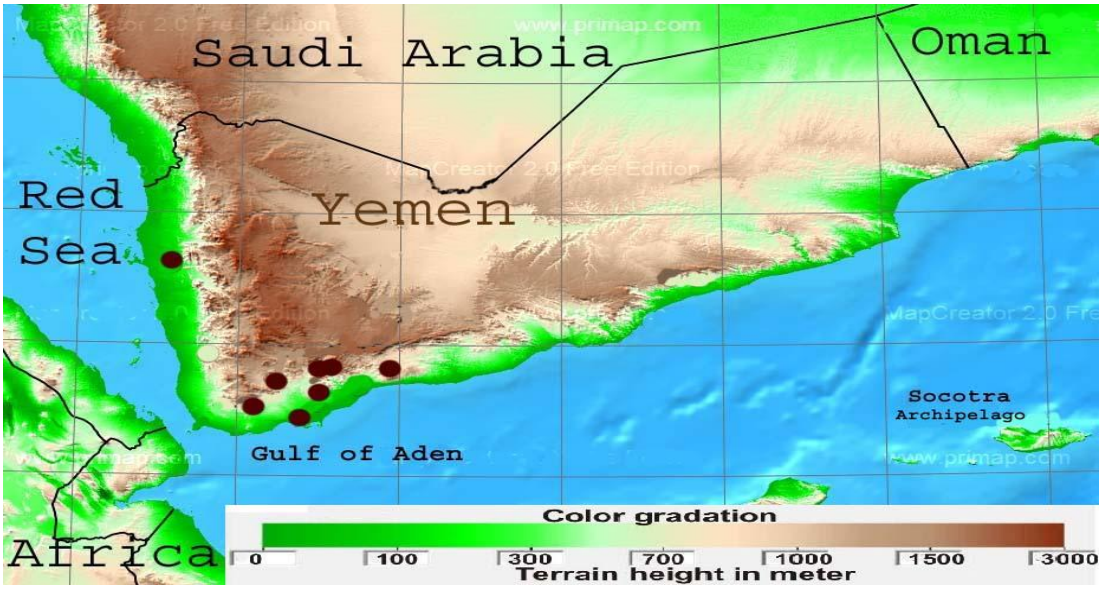


Figure 20: Distribution of *H. y. yerburii* in the mainland of Yemen.

Figure 21: typical specimen of *H. y. yerburii,* male, from Tour Al-Baha (NHM-BS N41859).

Description of undescribed species and one subspecies

Results revealed three *Hemidactylus* groups (group of *H. yerburii*, group of *H. robustus* and the new group of undescribed *Hemidactylus* species) which are present in the mainland in the phylogenetic trees of the mitochondrial genes.

Several clades within these groups refer to undescribed species. These undescribed species are represented in the clades of OTU 1 and OTU 3 from the *H. yerburii* group as well as the OTU 5, OTU 6 and OTU 7 from the group of new *Hemidactylus* species (fig. 13).

The group of *Hemidactylus yerburii* contains the members of the known subspecies of *H. y. yerburii* (OTU 2). However, the others are the undescribed units that are represented by the clades of OTU 1 and OTU 3. The description of OTU 1 fits to the description of the species *H. yerburii* with considerable variation, however, the members of OTU 3 clade have several distinct characters (see below).

The second group comprises the two known species of *H. robustus* (OTU 8) and *H. sinaitus* (OTU 4). Both are characterized above.

The third group consists of three clades not mentioned previously in the mainland or Socotra archipelago. The members of these clades consist of the OTU 5, OTU 6 and OTU 7.

Description of OTU 1 from the group of *H. yerburii*

This group contains three taxa referring to undescribed subspecies of *Hemidactylus yerburii* ssp. ‘ montanus’ (OTU 1), the known species of *Hemidactylus y. yerburii* (OTU 2) and the undescribed species of *Hemidactylus* sp. ‘ jumailiae’ (OTU 3).

1. ***Hemidactylus yerburii* ssp. ‘montanus’**

Materials: NHM-BS N41751 - NHM-BS N41756 from As-Sohool, Ibb; NHM-BS N41757 - NHM-BS N41770, NHM-BS N41772 - NHM-BS N41774, NHM-BS N41776 - NHM-BS N41779, NHM-BS N41785 NHM-BS N41794, NHM-BS N41796 - NHM-BS N41831 from Ibb, NHM-BS N41836 - NHM-BS N41838 from Al-Makhader, Ibb; NHM-BS N41839 from Jabal Rabbi, Ibb; NHM-BS N41840 - NHM-BS N41852 from Al-Odain, Ibb; NHM-BS N41771, NHM-BS N41775, NHM-BS N41780, NHM-BS N41795 from Yareem, Ibb; NHM-BS N41781 - NHM-BS N41784 from Mebar, Thamar; NHM-BS N41833 - NHM-BS N41834 from Wadah, Amran; NHM-BS N41835, NHM-BS N41853 from Sana’a. NHM-BS N41854 NHM-BS N41855 from Sana, Sana’a

Description of specimens:

Hemidactylus yerburii ssp. ‘montanus’ is a small to medium-sized gecko, with maximum recorded SVL of approximately 68 mm. Head sparsely covered with enlarged convex granules, the largest granules are between the eye and nostril. Nostril formed by the rostral, first upper labial and three nasals. 9 – 12 upper labials; 6 – 9 lower labials. Two pairs of post-mentals present, extending from the first lower labial shields into about the end of the second lower labials. Body depressed, covered with minute rounded granules with numerous large trihedral tubercles intermixed and

arranged mostly in 14 – 16 longitudinal rows; 74 – 97 dorsal scales across mid body. Ventral scales cycloid, imbricate, and larger than dorsals; 36 – 48 ventral scales across mid-abdomen. Limbs are rather short and thick. Digital pads moderately expanded; 5 – 7 lamellae under first toe, 9 – 11 lamellae under fourth toe. Tail slender, almost smooth dorsally, with a few small distinct tubercles; transverse row of six distinct tubercles; subcaudals uniform.

Dorsal coloration in some specimens is gray or brownish gray, with dusky band before and behind the eye, sometimes with feeble dusky markings on the head, neck and shoulders; on dorsal side pattern of a series of indistinct or distinct dark spots is present. On the middle of the tail towards the tip, there are faint or distinct indications of transverse dark bands. Ventral side is white, minutely spotted on the sides of the belly.

Differential Diagnosis

Several studies indicate that the differences between populations of *H. yerburii* in the high altitude of Yemen are due to the variation within the species since this species has extreme geographical variation (Arnold 1986), however, the results of phylogenetic trees revealed that OTU 1 is a separate sister clade of *H. y. yerburii* (OTU 2).

Following examination of the facial differences between the populations in the group of *H. yerburii*, results revealed that the population of OTU 1 (*Hemidactylus yerburii* ssp. ‘montanus’) can be distinguished from the population of OTU 2 (*H. y. yerburii*) by the low mean number of dorsal scales (87.19 vs. 91.70 in males and 85.40 vs. 91.70 in females), the low mean number of scansors under the first toe (6.26 vs. 6.70

in males and 6.25 vs. 6.83 in females) and the low mean number of the male pores (10.19 vs. 12.50). Furthermore, the members of OTU 1 are relatively smaller than OTU 2.

The members of undescribed subspecies of OTU 1 differ from the members of OTU3 (*Hemidactylus* sp. 'jumailiae') by the high mean number of ventral scales (42.45 vs. 35.50 in females), the high mean number of dorsal scales (87.19 vs. 65.50 in males and 85.40 vs. 67.08 in females), the high mean number of tubercle dorsal scales (15.09 vs. 12.00 in males and 15.47 vs. 12.75 in females), the high mean number of internasal granules (1.00 vs. 0.50 in males and 1.00 vs. 0.75 in females), the low mean number of scansors under the first toe (6.25 vs. 7.00 in males), the low mean number of scansors under the fourth toe (10.19 vs. 11.00 in males) and the high mean number of male pores (10.19 vs. 7.50) (table 10, 13).

Since there is only one male specimen in the members of OTU 4 (*H. sinaitus*), the comparison between the members of OTU 1 and OTU 4 will be among the females.

OTU 1 differs from the OTU 4 by the high mean number of ventral scales (42.45 vs. 35.00), the high mean number of dorsal scales (85.40 vs. 73.00), the high mean number of tubercle dorsal scales (15.47 vs. 14.20), the high mean number of upper labials (10.37 vs. 8.80 in females), the high mean number of lower labials (7.83 vs. 7.00), the high mean number of scansors under the first toe (6.25 vs. 5.00) and the high mean number of scansors under the fourth toe (10.10 vs. 9.00). In general, the snout-vent-length in OTU 1 is higher than OTU 4.

The members of OTU 1 differ from the members of OTU 5 (*Hemidactylus* sp. 'shihraensis') by the low mean number of ventral scales (40.91 vs. 52.50 in males

and 42.45 vs. 47.50 in females), the high mean number of dorsal scales (87.19 vs. 78.00 in males and 85.40 vs. 70.00 in females), the high mean number of tubercle dorsal scales (15.09 vs. 13.00 in males and 15.47 vs. 14.00 in females) and the high mean number of male pores (10.19 vs. 6.00).

The members of OTU 1 differ from the members of OTU 6 (*Hemidactylus* sp. 'saba') by the high mean number of ventral scales (40.91 vs. 31.00 in males and 42.45 vs. 30.00 in females), the high mean number of dorsal scales (87.19 vs. 76.50 in males), the high mean number of tubercle dorsal scales (15.09 vs. 14.00 in males and 15.47 vs. 14.00 in females), the high mean number of upper labials (10.31 vs. 8.50 in males and 10.37 vs. 9.00 in females), the low mean number of scansors under the first toe (6.25 vs. 8.00 in males as well as in females), the low mean number of scansors under the fourth toe (10.19 vs. 11.00 in males and 10.10 vs. 11.00 in females) and the high mean number of male pores (10.19 vs. 6.00).

Though there is only one female specimen of the OTU 7 (*Hemidactylus* sp. 'ulii'), but considerable differences are recognized between OTU 1 and OTU 7 in the high numbers of dorsal scales (85.40 vs. 63.00), the high numbers of upper labials (10.37 vs. 8), the high number of scansors under the first toe (6.25 vs. 5.00) and the high number of scansors under the fourth toe (10.10 vs. 8.00).

The members of OTU 1 differ from the members of OTU 8 (*H. robustus*) in females by the high mean number of ventral scales (42.45 vs. 37.80), the high mean number of dorsal scales (85.40 vs. 70.80) and the high mean number of upper labials (10.37 vs. 8.60) (table 10).

The undescribed subspecies (*H. yerburii* ssp. ‘montanus’) differs from *H. lemurinus* by the distinct character of dorsal tubercle. There are no dorsal tubercles in *H. lemurinus*. Furthermore, the number of male pores is less in *H. lemurinus*.

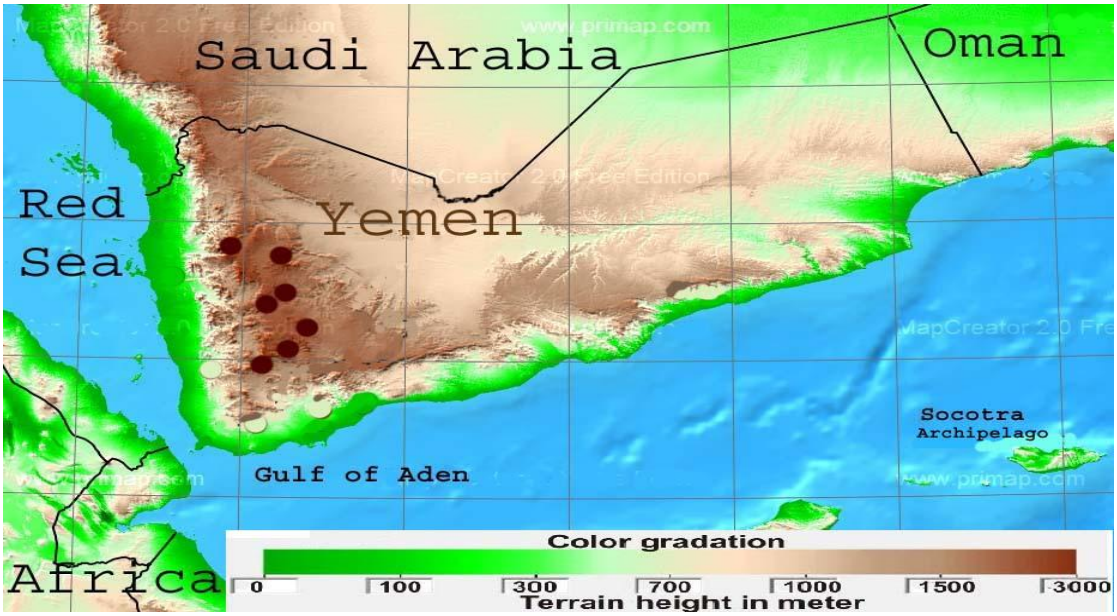


Figure 22: Distribution of *Hemidactylus yerburii* ssp. in the mainland of Yemen.

Figure 23: typical specimen of undescribed *Hemidactylus yerburii* ssp. 'montanus' female, from Al-Makhader, Ibb (NHM-BS N41836).

Description of OTU 3 from the group of *H. yerburii*

2. *Hemidactylus* sp. 'jumailiae'

Materials: NHM-BS N41889 - NHM-BS N41890, NHM-BS N41893- NHM-BS N41897 from Ibb; NHM-BS N41892, NHM-BS N41900 - NHM-BS N41901 from Sana'a; NHM-BS N41898 NHM-BS N41899 from Thamar.

Description of specimens:

Hemidactylus sp. 'jumailiae' is a small to medium-sized gecko, with maximum recorded SVL of approximately 47 mm. Head moderately high. Nostril formed by the rostral, boarded by first upper labial and three nasals. 8 – 12 upper labials; 7 – 9 lower labials. Two pairs of post-mentals present, extending from the first lower labial shields into half of the second lower labials. Body depressed, covered with minute rounded granules with numerous small cycloid tubercles intermixed and arranged mostly in 10 – 14 longitudinal rows; 62 – 80 dorsal scales across mid body. Ventral scales rhomboid, imbricate, and larger than dorsals; 31 – 42 ventral scales across mid-abdomen. Limbs are rather short and thick. Digital pads moderately expanded; 6 – 7 lamellae under first toe, 9 – 12 lamellae under fourth toe. Males with 6 – 9 preanal pores. Tail is transverse, almost smooth dorsally, with a few small distinct tubercles; transverse row of eight distinct tubercles; subcaudals uniform.

General dorsal color of most specimens is pale brown to light brownish gray, pattern usually made of a series of distinct brown spots arranged transversely along mid-dorsum. Pair of brown lines on each side of the head extending from the nasals until occipital side. Tail with several dark or light brown bands, occasionally with indistinct brown spots.

Differential Diagnosis

The members of OTU 3 (*Hemidactylus* sp. 'jumailiae') can be distinguished from the *Hemidactylus y. yerburii* by the low mean number of ventral scales (35.50 vs. 41.35 in females), the low mean number of dorsal scales (65.50 vs. 91.70 in males and 67.08 vs. 91.30 in females), the low mean number of tubercle dorsal scales (12.00 vs. 15.40 in males and 12.75 vs. 15.30 in females), the low mean number of scansors under the first toe (6.33 vs. 6.83 in females) and the low mean number of the male pores (7.50 vs. 12.50) (table 10, 13).

They differ from the members of undescribed subspecies OTU 3 (*Hemidactylus yerburii* ssp. 'montanus') by the low mean number of ventral scales (35.50 vs. 42.45 in females), the low mean number of dorsal scales (65.50 vs. 87.91 in males and 67. 08 vs. 85.40 in females), the low mean number of tubercle dorsal scales (12.00 vs. 15.09 in males and 12.75 vs. 15.47 in females), the low mean number of internasal granules (0.50 vs. 1.00 in males and 0.75 vs. 1.00 in females), the high mean number of scansors under the first toe (7.00 vs.6.25 in males), the high mean number of scansors under the fourth toe (11.00 vs. 10.19 in males) and the low mean number of male pores (7.50 vs. 10.19).

The members of OTU 3 differ from the OTU 4 by the low mean number of tubercle dorsal scales (12.75 vs. 14.20), the high mean number of upper labials (10.00 vs. 8.80 in females), the high mean number of lower labials (8.08 vs. 7.00), the low mean number of internasal granules (0.75 vs. 1.00 in females), the high mean number of scansors under the first toe (6.33 vs. 5.00) and the high mean number of scansors

under the fourth toe (10.17 vs. 9.00). In general, snout-vent-length in OTU 1 is higher than OTU 4 (table 10).

The members of OTU 3 (*Hemidactylus yerburii* ssp. 'montanus') differ from the undescribed species of OTU 5 (*Hemidactylus* sp. 'shihraensis') by the low mean number of ventral scales (38.00 vs. 52.50 in males and 35.50 vs. 47.50 in females), the low mean number of dorsal scales (65.50 vs. 78.00 in males), the low mean number of tubercle dorsal scales (12.00 vs. 13.00 in males and 12.75 vs. 14.00 in females), the low mean number of internasal granules (0.50 vs. 1.00 in males and 0.75 vs. 1.00 in females) and the high mean number of male pores (7.50 vs. 6.00).

The members of OTU 3 differ from the members of undescribed species of OTU 6 (*Hemidactylus* sp. 'saba') by the high mean number of ventral scales (38.00 vs. 31.00 in males and 35.50 vs. 30.00 in females), the low mean number of dorsal scales (65.50 vs. 76.50 in males and 67.08 vs. 80.00 in females), the low mean number of tubercle dorsal scales (12.00 vs. 14.00 in males and 12.75 vs. 14.00 in females), the low mean number of internasal granules (0.50 vs. 1.00 in males and 0.75 vs. 1.00 in females), the low mean number of scansors under the first toe (7.00 vs. 8.00 in males and 6.33 vs. 8.00 in females) and the high mean number of male pores (7.50 vs. 6.00).

There is only one female specimen of the undescribed species of OTU 7 (*H.* sp. 'ulii'), but considerable differences are recognized between this undescribed species and OTU 7 by the high number of upper labials (10.00 vs. 8), the high number of scansors under the first toe (6.33 vs. 5.00) and the high number of scansors under the fourth toe (10.17 vs. 8.00).

The members of the undescribed species (*Hemidactylus* sp. ‘jumailiae’) differ from the members of OUT 8 (*H. robustus*) by the low mean number of tubercle scales (12.75 vs. 15.60) and the high mean number of upper labials (10.00 vs. 8.60) (table 10).

It also differs from *H. lemurinus* recorded in the mainland by the distinct character of dorsal tubercles. There are no dorsal tubercles in *H. lemurinus*, furthermore, the male pores are less in *H. lemurinus* than in this undesribed species, and the number of scansors under the fourth toe is lower in the *H.* sp. ‘jumailiae’.

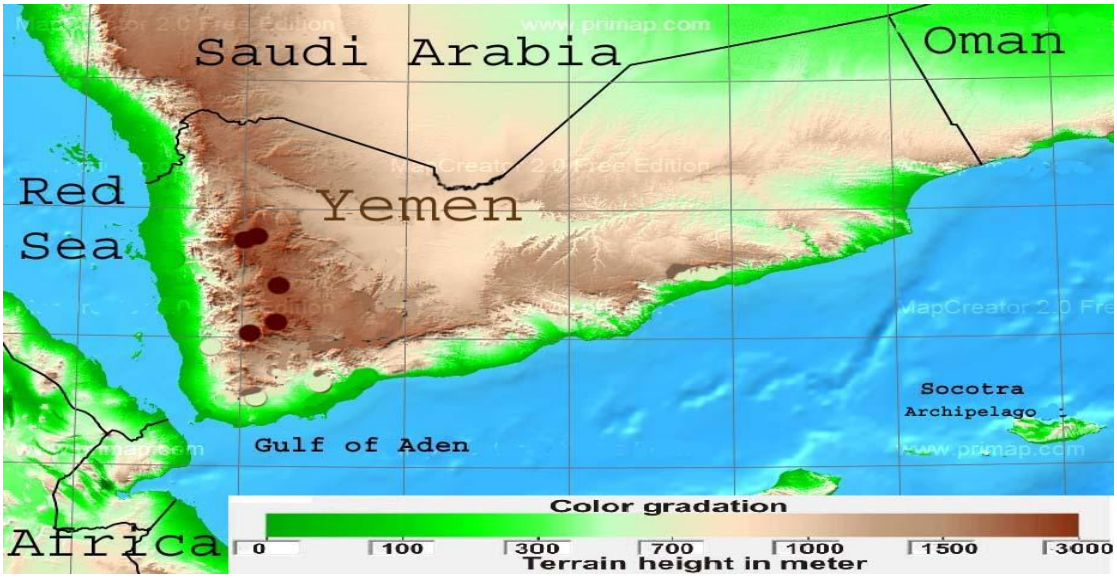


Figure 24: Distribution of *Hemidactylus* sp. 'jumailiae' in the mainland of Yemen.

Figure 25.: typical specimen of undescribed *Hemidactylus* sp.'Jumailiae', male from Ibb.

The group of *H. robustus*

This group is found in the coastal plain of the mainland and consists of the two monophyletic clades of OTU 8 assigned to the species of *H. robustus* and OTU 4 determined as *H. sinaitus* (described previously).

The members of *Hemidactylus robustus* can be distinguished from *H. sinaitus* by the high mean number of tubercle scales (15.60 vs. 14.20 in females), the high mean number of scansors under the first toe (6.20 vs. 5.00 in females) also the high mean number of scansors under the fourth toe (9.80 vs. 9.00 in females) (table 10, 14).

The group of undescribed *Hemidactylus* species

This group in the mainland consists of the clade of OTU 5 from the coastal plain of Yemen, the clade of OTU 6 from the desert, and the one specimen (OTU 7) from the high mountains. The members of these clades represent undescribed taxa of *Hemidactylus* (fig. 13).

Description of OTU 5 from the group of undescribed *Hemidactylus* species

3. *Hemidactylus* sp. 'shihraensis'

Materials: NHM-BS N41908 - N41909 from Ash-Shihr, NHM-BS N41910 - N41911 from Ghail Bawzeer.

Description of specimens:

In general, they are small to medium-sized geckos, with maximum recorded SVL of 48.2 mm. Head moderately high. Nostril bordered by rostral, three nasals and mostly the first upper labials not in contact with upper nasals. 9 – 10 upper labials; 7 – 8 lower labials. Two pairs of post-mentals present, extending from the first lower labial shields into about the half of the second lower labials. Dorsal scales granular and small, 69 – 74 dorsal scales; dorsal tubercles large, keeled, arranged in 14 longitudinal rows across mid body. 46 – 54 ventral scales across mid-abdomen. Limbs are rather short and thick. Digital pads moderately expanded; six lamellae under first toe, ten lamellae under fourth toe. Males with six pre-anal pores. Tail slender, almost smooth dorsally, with a few small distinct tubercles; transverse row of six tubercles; subcaudals uniform.

The general color of the specimens is pinkish brown to light yellowish brown, with series of regular indistinct brown cross-bars extending somewhat on the dorsum to the end of the tail.

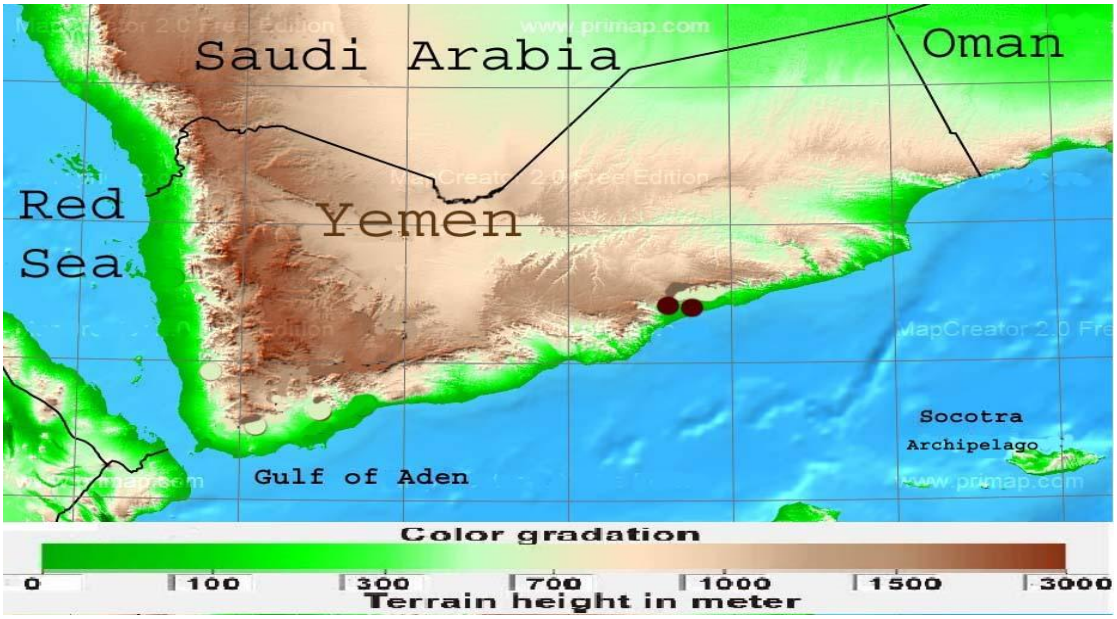


Figure 26: Distribution of *Hemidactylus* sp. 'shihraensis' in the mainland of Yemen.

Figure 27: typical specimen of undescribed *Hemidactylus* sp. 'shihraensis', from Ghail Bawzeer, Hadhramout (NHM-BS N41910).

Differential Diagnosis of the undescribed species in this group.

The undescribed species of OTU 5 (*Hemidactylus* sp. 'shihraensis') can be distinguished from the members of undescribed species of OTU 6 (*Hemidactylus* sp. 'saba') by the higher mean number of ventral scales (52.50 vs. 31.00 in males and 47.50 vs. 30.00 in females), the higher mean number of upper labials (9.50 vs. 8.50 in males), the lower mean number of scansors under the first toe (6.00 vs. 8.00 in males as well as in females) also the lower mean number of scansors under the fourth toe (10.00 vs. 11.00 in males as well as in females) (table 8, 13).

Both populations of OTU 5 (*Hemidactylus* sp. 'shihraensis') and OTU 6 (*Hemidactylus* sp. 'saba') have larger numbers of scansors under the first and fourth toe than the specimen of OTU 7 (*Hemidactylus* sp. 'ulii'). The single specimen of OTU7 has only five scansors under the first toe and eight scansors under the fourth toe. Furthermore, the number of ventral scales in OTU 7 is lower than the number in OTU 5 but larger than OTU 6. Moreover, the number of dorsal scales is lower than both populations of OTU 5 and OTU 6.

The differences among the undescribed species of *H.* sp. 'shihraensis' and the members of OTU 1 (*H. yerburii* ssp. 'montanus') and OTU 3 (*H.* sp. 'jumailiae') were described above.

The members of OTU 5 differ from the OTU 2 (*H. y. yerburii*) by the high mean number of ventral scales (52.50 vs. 41.00 in males and 47.50 vs. 41.35 in females), the low mean number of dorsal scales (78.00 vs. 91.70 in males and 70.00 vs. 91.30 in females), the low mean number of tubercle dorsal scales (13.00 vs. 15.40 in males

and 14.00 vs. 15.30 in females) and the low mean number of male pores (6.00 vs. 12.50) (table 10).

The members of OTU 5 differ from the OTU 4 (*H. sinaitus*) by the high mean number of ventral scales (47.50 vs. 35.00 in females), the high mean number of scansors under the first toe (6.00 vs. 5.00 in females) and the high mean number of scansors under the fourth toe (10.00 vs. 9.00 in females). However there is only one specimen of the OTU 7 (*Hemidactylus* sp. 'ulii'), but considerable differences are recognized between OTU 5 and OTU 7 in the high numbers of upper labials (9.50 vs. 8) and the high number of scansors under the fourth toe (10.00 vs. 8.00).

The members of OTU 5 differ from the members of OTU 8 (*H. robustus*) in females by the low mean number of tubercle scales (12.75 vs. 15.60) and the high mean number of upper labials (10.00 vs. 8.60). The new species *H. shihraensis* sp. nov. differs from *H. lemurinus* by the distinct character of dorsal tubercle. There are no dorsal tubercles in *H. lemurinus*, furthermore, the number of scansors under the fourth toe is lower in the new species than *H. lemurinus*.

The members of OTU 6 (*Hemidactylus* sp. 'saba') differ from the OTU 4 (*H. sinaitus*) by the low mean number of ventral scales (30.00 vs. 35.00 in females), the high mean number of dorsal scales (80.00 vs. 73.00 in females), the high mean number of scansors under the first toe (8.00 vs. 5.00 in females) and the high mean number of scansors under the fourth toe (11.00 vs. 9.00 in females). The members of OTU 6 differ from the OTU 2 (*H. y. yerburii*) by the low mean number of ventral scales (31.00 vs. 41.00 in males and 30.00 vs. 41.35 in females), the low mean number of dorsal scales (76.50 vs. 91.70 in males and 80.00 vs. 91.30 in females),

the low mean number of tubercle dorsal scales (14.00 vs. 15.40 in males and 14.00 vs. 15.30 in females), the high mean number of scansors under the first toe (8.00 vs. 6.70 in males and 8.00 vs. 6.83 in females) and the low mean number of male pores (6.00 vs. 12.50) (table 8 and 12). Though there is only one specimen of the OTU 7 (*Hemidactylus* sp. 'ulii'), but considerable differences are recognized between OTU 6 and OTU 7 in the low number of ventral scales (30.00 vs. 41.00), the high numbers of dorsal scales (80.00 vs. 68.00), the high number of scansors under the first toe (8.00 vs. 5.00) and the high number of scansors under the fourth toe (11.00 vs. 8.00). The members of OTU 6 differ from the members of OTU 8 (*H. robustus*) in females by the low mean number of ventral scales (30.00 vs. 37.80), the low mean number of tubercle scales (14.00 vs. 15.60) and the high number of scansors under the first toe (8.00 vs. 6.20) and the high number of scansors under the fourth toe (11.00 vs. 9.80). The difference of the single specimen of OTU 7 (*Hemidactylus* sp. 'ulii') and the other species was described above.

The undescribed species (*Hemidactylus* sp. 'saba') differs from *H. lemurinus* by the distinct character of dorsal tubercles. There are no dorsal tubercles in *H. lemurinus*, furthermore, the number of scansors under the first toe is higher in the new species than in *H. lemurinus*. However, the undescribed species of OTU 7 (*Hemidactylus* sp. 'ulii') differs from *H. lemurinus* by the distinct character of dorsal tubercles. There are no dorsal tubercles in *H. lemurinus*, furthermore, the number of scansors under the first and fourth toe is lower in the new species than in *H. lemurinus*.

Description of OTU 6 from the group of undescribed *Hemidactylus* species

4. *Hemidactylus* sp. 'saba'

Materials: NHM-BS N41912 – N41913 from Al-Abr - NHM-BS N41914 from Al-Mojamma, NHM-BS N41915 from Wadi Al-Jufair, Mareb.

Description of specimens

Hemidactylus sp. 'saba' is a small to medium-sized gecko, with maximum recorded SVL of approximately 59 mm. Head moderately high. Nostril bordered by rostral, three nasals and mostly the first upper labials in contact with upper nasals. 8 - 9 upper labials; 7 – 8 lower labials. Two pairs of post-mentals present, extending from the first lower labial shields into about the half of the second lower labials. Dorsal scales are granular and small, 76 – 82 dorsal scales; dorsal tubercles large, weakly keeled, arranged in 14 longitudinal rows across mid body. 28 – 32 ventral scales across mid-abdomen. Limbs are rather short and thick. Digital pads moderately expanded; eight lamellae under first toe, 11 lamellae under fourth toe. Males with six pre-anal pores. Tail slender, almost smooth dorsally, with a few small distinct tubercles; transverse row of eight tubercles; subcaudals uniform. Basic dorsal color is brownish gray with regular dark bands, sometimes with irregular indistinct dark mottling, extending somewhat to the beginning of the tail. Tail is light brown with indistinct dark bands. The specimens were found during the day on old buildings.

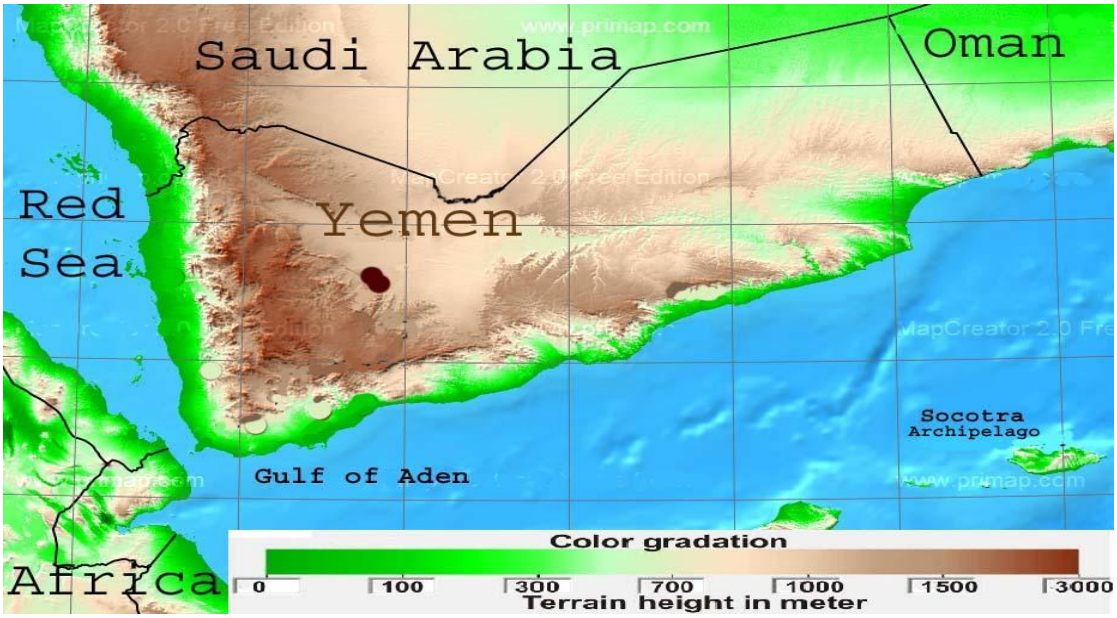


Figure 28: Distribution of *Hemidactylus* sp. 'saba' in the mainland of Yemen.

Figure 29: typical specimen of undescribed *Hemidactylus* sp. 'saba', from Al-Abr, Mareb (NHM-BS N41914).

Description of OTU 7 from the group of undescribed *Hemidactylus* species

5. *Hemidactylus* sp. 'ulii'

Material: NHM-BS N41912 male from Radman, Lahj, W. Mustafa, 13. 11.2007.

Description of specimen:

Snout-vent length: 32.4 mm. Head length: 10.4 mm. Head width: 6.2 mm. Head height: 3.7 mm. upper labials: 8. Lower labials: 8. Rows of dorsal tubercles: 14, keeled, dorsal scales across mid-body 76. Ventral scales across mid-abdomen 47. Lamellae under fourth toe: 7. Lamellae under first toe: 5. Nostril surrounded by 3 nasals, rostral and the first upper labial. Internasal granules separated by one scale. Mental large, sub-triangular. Anterior postmental nearly as wide as long, shorter than mental, expanded into more the half of second lower labials. Tail is slender, rather depressed, tubercles on tail rather flat, weakly keeled, restricted, arranged in eight rows.

The general dorsal color is yellowish brown with two clear dark bands then becomes pale toward tail, extending somewhat to the beginning of the tail. Tail is yellowish brown with five distinct dark bands.

Unfortunately, there is only one specimen of OTU 7 collected throughout this study, therefore, the morphological tests were not applied to this single specimen. However, the phylogenetic mitochondrial and nuclear trees and the genetic divergence among the populations in this group confirm the differences among the members of this group (more details in the phylogenetic discussion).

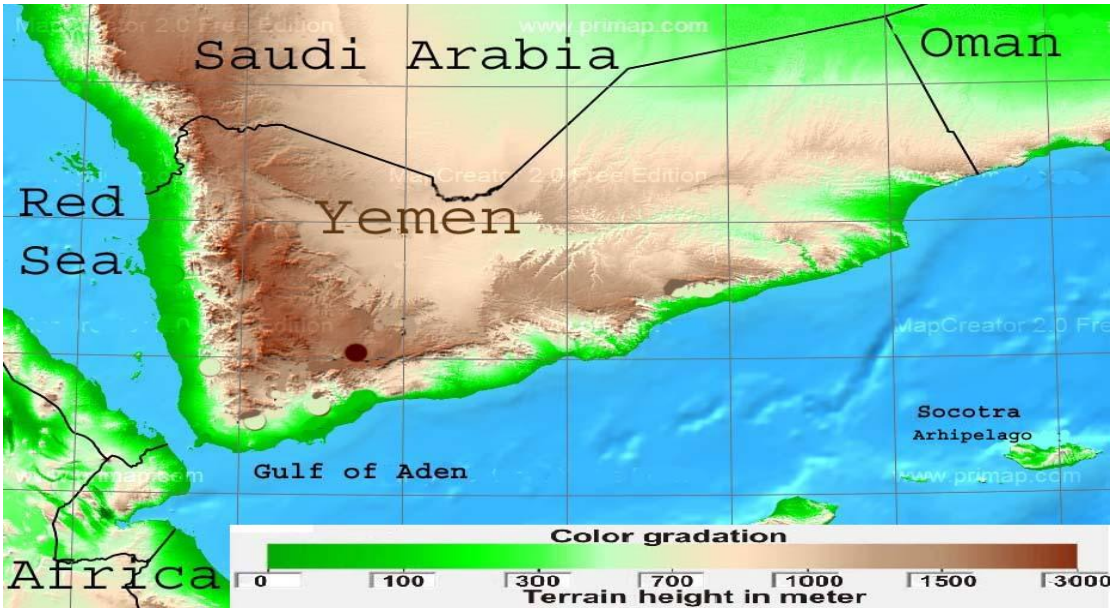


Figure 30: Distribution of *Hemidactylus* sp. ('ulii') in the mainland of Yemen.

Figure 31: Only specimen of undescribed *Hemidactylus* sp. 'ulii' from Radman, Al-Baidha (NHM-BS N41912).

Conclusions

This study highlights the extraordinary diversity of *Hemidactylus* in the mainland of Yemen and findings confirm that cryptic species are present in this genus in Yemen. The results strengthen the possibility of sympatric and allopatric speciation in this area.

The present study indicates that there are at least ninth taxa of known *Hemidactylus* species and undescribed species found in the mainland: *Hemidactylus flaviviridis*, *H.* sp. 'jumailiae', *H. lemurinus*, *H. robustus*, *H.* sp. 'saba', *H. sinaitus*, *H.* sp. 'shihraensis', and subspecies of *H. y. yerburii* and *H. yerburii* ssp. 'montanus'. A tenth species *H.* sp. 'ulii' may be distinguishable, but needs further collections.

The occurrence of *H. sinaitus* in Aden and Lahj area is due to accidental introduction; this also, may be the case of *H. robustus*. The geckos of *H.* sp. 'jumailiae', *H.* sp. 'saba', *H.* sp. 'shihraensis' and the undescribed subspecies of *H. yerburii* ssp. 'montanus' may represent endemic species to the mainland.

It is obvious throughout the study that the species *H. homoeolepis* occurs only in Socotra archipelago, and there is doubt of the existence of this species in the mainland. Additional field work is needed in the area of east and north Yemen near the boundary of Saudi Arabia and Oman.

In the case of *H. lemurinus*, this species recorded from Wadi Hajir and Sayhut in Hadhramout and Al-Mahra governorates. Also, it may occur in the east of Al-Mahra governorates in the eastern part of Yemen as an extension to existence in Dhofar (Oman).

The recording of *H. persicus* is unreliable since this species is found in the north-east Arabia as well as the mountain regions in north-east Oman. Therefore, the distribution to the eastern region in Yemen is irrational since several natural barriers will prevent its extension.

The occurrence of the Turkish house gecko *H. turcicus* which was recorded previously in the mainland of Yemen is inaccurate. This mistake could not be avoided because of the considerable variation in the range of external characters among species as the previous studies on these geckos depended only on morphological characters.

Additional field work is needed in the area of east and north Yemen near the boundary of Saudi Arabia and Oman. Furthermore, the populations of *Hemidactylus* gecko from Najran in Saudi Arabia are an interesting subject to study in order to confirm their taxonomic status.

In general, the study of herpetological species in Yemen is far from complete, and more additional field work and molecular studies are necessary to arrive to a more accurate picture of the true status of species in Yemen.

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Summary

Yemen has a highly specialized fauna and flora of peculiar interest to taxonomic researchers and to evolutionary biologists. Yemen contains a variety of habitats which range from coastal mangroves, shrub lands and dunes along the coastal plains to the eastern deserts and an array of mountain habitats that reach elevations around 3666 m at the tip of the mountain of Jabel Al-Nabi Shauib, the highest point in the Arabian Peninsula. This unique geographical position at the junction point of the Red sea and Arabian Sea has given Yemen different climatic features and topographic areas. These features are favorable for the existence of diverse ecosystems along with a high level of biodiversity, in that way the country has a rich and diverse fauna and flora.

There is still need for systematics and phylogenetic studies for the classification of reptiles in Yemen as well as other animals. For that reason, this study is important to clarify the taxonomic status of the genus *Hemidactylus* in the mainland of Yemen. This study depends on both the morphological and molecular methods.

The aim of this research is to identify specimens of *Hemidactylus* geckos collected from the mainland of Yemen using morphological and molecular approaches.

Hemidactylus Oken, 1817 is widely distributed and considered one of the most species-rich genera of the family Gekkonidae. The recent information on the status of this genus is presented based on individuals of these geckos collected during the period 2007 to 2009 from several localities in the mainland. Morphometric and

meristic data were included in discriminant function analyses (DFA) and principal component analyses (PCA) using the analysis program of SPSS for windows, version 18. To assess significant differences among taxa One-Way-ANOVA test and Independent-Samples T-test were performed. These results were confirmed by using the test of Mann-Whitney (U-test).

The phylogenetic study of Yemeni *Hemidactylus* was based on 1465 base pairs of mitochondrial genes (736 bp cytochrome *b* and 370 bp 12S rRNA) and one nuclear gene (359 bp phosducin) from specimens of *Hemidactylus* in addition to several sequences from known species of *Hemidactylus* obtained from the Genbank. Two sequences of *Hemidactylus angulatus* from Niger were added as an outgroup, in addition to tissues extracted from known Socotran species obtained and classified previously by Prof. Dr. Ulrich Joger were analyzed throughout the study. The phylogenetic analysis clearly distinguishes eight monophyletic taxa of *Hemidactylus* in the mainland which can be assigned to three groups: three taxa are members of the *H. yerburii* group, two clades are members of the *H. robustus* group and three compose a new group of undescribed species.

The present study indicates that there are ten taxa of known *Hemidactylus* species and undescribed species found in the mainland: *Hemidactylus flaviviridis*, *H.* sp. 'jumailiae', *H. lemurinus, H. robustus, H.* sp. 'saba', *H. sinaitus, H.* sp. 'shihraensis', *H.* sp. 'ulii' and two subspecies of *H. yerburii*: *H. y. yerburii* and *H. yerburii* ssp. 'montanus'.

The existence of *H. turcicus*, *H. persicus* and *H. homoeolepis* are discussed throughout the study. There is considerable evidence that *H. turcicus* has to be erased from the list of Yemeni mainland species. Further studies are required in the northeastern and eastern Yemen to confirm the existence of remaining species.

Zusammenfassung

Jemen hat eine hoch spezialisierte Fauna und Flora von besonderem Interesse für taxonomische Forschung und Evolutionsbiologie. Jemen umfasst eine Vielzahl von Lebensräumen, diese reichen von den Küsten-Mangroven, Buschland und Dünen entlang der Küstenebenen zu den östlichen Wüsten und einer Reihe montaner Lebensräume rund 3666 m ist der Jabel Al-Nabi Shauib der höchste Punkt der Arabischen Halbinsel. Diese einzigartige geographische Lage zwischen Verbindungspunkt des Rotem Meer und Arabischem Meer ist günstig für die Existenz verschiedener Ökosysteme zusammen mit einer hohen Artenvielfalt, auf diese Weise das Land hat eine reiche und vielfältige Fauna und Flora.

Diese Arbeit soll einen Beitrag zur Klassifizierung von Reptilien im Jemen liefern, wobei sie sich genauer mit dem taxonomischen Status der Gattung *Hemidactylus* unter Anwendung morphologischer und molekularer Methoden beschäftigt.

Das Ziel dieser Arbeit ist die Erforschung und Klassifizierung der Geckos der Gattung *Hemidactylus* in Jemen.

Hemidactylus Oken, 1817 bilden eine artenreiche Gattung und gehören zur Familie der Geckos (Gekkonidae). Von gesammelten wurden morphometrische und meristische Daten erfasst und mittels Diskriminanzanalyse (DFA) und Hauptkomponentenanalyse (PCA) analysiert.

Um signifikante Unterschiede zwischen den Taxa feststellen zu können, wurden One-Way-ANOVA-Tests und T-Tests für unabhängige Stichproben durchgeführt

sowie zusätzlich zur Bestätigung der Ergebnisse der Test von Mann-Whithney (U-Test).

Schließlich lieferten 1465 Basenpaare mitochondrialer Gene (736 bp Cytochrom b und 370 bp 12 S rRNA) und ein Zellkern-Gen (359 bp Phosducin) aus Proben der Halbfinger-Geckos unter Berücksichtigung bereits vorhandener Sequenzen bekannter Arten aus der Genbank, die Grundlage für die molekulare Phylogenie der jemenitischen Geckos. Darüber hinaus wurden zwei Sequenzen von *Hemidactylus angulatus* aus Niger und Gewebsproben bekannter Arten der Inselgruppe Sokotra, welche Professor Dr. Ulrich Joger zuvor klassifiziert hat, in dieser Studie mitberücksichtigt. Die phylogenetischen Analysen unterscheiden eindeutig acht monophyletische Taxa von *Hemidactylus* auf dem Festland, welche drei Gruppen zugeordnet werden können: Drei Taxa stammen aus der Gruppe *H. yerburii*, zwei aus der Gruppe *H. robustus* und drei bilden eine Gruppe bisher unbekannter Spezies.

Die vorliegende Studie zeigt, dass es im Jemen insgesamt zehn Taxa bekannter *Hemidactylus*-Arten und unbeschriebener Arten gibt, die auf dem Festland gefunden wurden: *Hemidactylus flaviviridis, H.*sp. 'jumailiae', *H. lemurinus, H. robustus, H.* sp. 'saba', *H. sinaitus, H.* sp. 'shihraensis', *H.* sp. 'ulii' und zwei Subspezies von *H. yerburii*: *H. y. yerburii* und *H. yerburii* ssp. 'montanus'.

Die Thematik um von Existenz der *H. turcicus, H. persicus* und *H. homoeolepis* in Jemen wurde während der gesamten Studie behandelt, jedoch lassen die Ergebnisse den Schluss zu, dass *H. turcicus* aus der Liste der jemenitischen Festland-Arten gelöscht werden muss. Weitere Studien sind im nordöstlichen und östlichen Jemen erforderlich, um die Existenz der übrigen Arten zu bestätigen.

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Appendices

Appendix I: list of samples deposited in Natural History Museum, Braunschweig, Germany.

No.	*Code No.*	*Museum No.*	*OTU*	*Genus*	*locality*	*Date of Coll.*
1	SBR 020	N41751	1	*H. yerburii* ssp.	Ibb	22.11.2007
2	SBR 021	N41752	1	*H. yerburii* ssp.	Ibb	06.11.2007
3	SBR 023	N41753	1	*H. yerburii* ssp.	Ibb	25.12.2007
4	SBR 024	N41754	1	*H. yerburii* ssp.	Ibb	25.12.2007
5	SBR 025	N41755	1	*H. yerburii* ssp.	Ibb	25.12.2007
6	SBR 026	N41756	1	*H. yerburii* ssp.	Ibb	19.11.2007
7	SBR 027	N41757	1	*H. yerburii* ssp.	Ibb	20.11.2007
8	SBR 028	N41758	1	*H. yerburii* ssp.	Ibb	20.11.2007
9	SBR 031	N41759	1	*H. yerburii* ssp.	Thamar	13.10.2007
10	SBR 037	N41760	1	*H. yerburii* ssp.	Ibb	30.10.2007
11	SBR 038	N41761	1	*H. yerburii* ssp.	Ibb	30.10.2007
12	SBR 039	N41762	1	*H. yerburii* ssp.	Ibb	30.10.2007
13	SBR 040	N41763	1	*H. yerburii* ssp.	Ibb	11.11.2007
14	SBR 041	N41764	1	*H. yerburii* ssp.	Ibb	18.11.2007
15	SBR 042	N41765	1	*H. yerburii* ssp.	Ibb	11.11.2007
16	SBR 043	N41766	1	*H. yerburii* ssp.	Ibb	12.11.2007
17	SBR 044	N41767	1	*H. yerburii* ssp.	Ibb	19.12.2007
18	SBR 045	N41768	1	*H. yerburii* ssp.	Ibb	24.11.2007
19	SBR 046	N41769	1	*H. yerburii* ssp.	Ibb	12.11.2007
20	SBR 047	N41770	1	*H. yerburii* ssp.	Ibb	10.11.2007
21	SBR 048	N41771	1	*H. yerburii* ssp.	Yareem	10.11.2007
22	SBR 049	N41772	1	*H. yerburii* ssp.	Yareem	10.11.2007
23	SBR 050	N41773	1	*H. yerburii* ssp.	Ibb	10.11.2007
24	SBR 051	N41774	1	*H. yerburii* ssp.	Yareem	10.11.2007
25	SBR 052	N41775	1	*H. yerburii* ssp.	Yareem	10.11.2007
26	SBR 053	N41776	1	*H. yerburii* ssp.	Ibb	12.11.2007
27	SBR 054	N41777	1	*H. yerburii* ssp.	Yareem	20.11.2007
28	SBR 055	N41778	1	*H. yerburii* ssp.	Ibb	11.11.2007
29	SBR 059	N41779	3	*Hemidactylus* sp.	Ibb	07.12.2007

Continuation of Appendix I.

30	SBR 060	N41780	3	*Hemidactylus* sp.	Yareem	11.11.2007
31	SBR 061	N41781	1	*H. yerburii* ssp.	Ibb	11.11.2007
32	SBR 062	N41782	3	*Hemidactylus* sp.	Ibb	11.11.2007
33	SBR 063	N41783	1	*H. yerburii* ssp.	Ibb	11.11.2007
34	SBR 064	N41784	1	*H. yerburii* ssp.	Ibb	25.12.2007
35	SBR 065	N41785	1	*H. yerburii* ssp.	Ibb	25.12.2007
36	SBR 066	N41786	1	*H. yerburii* ssp.	Ibb	25.12.2007
37	SBR 067	N41787	1	*H. yerburii* ssp.	Ibb	25.12.2007
38	SBR 068	N41788	1	*H. yerburii* ssp.	Ibb	25.12.2007
39	SBR 069	N41789	3	*Hemidactylus* sp.	Yareem	24.11.2007
40	SBR 072	N41790	3	*Hemidactylus* sp.	Yareem	24.11.2007
41	SBR 074	N41791	1	*H. yerburii* ssp.	Ibb	12.11.2007
42	SBR 075	N41792	1	*H. yerburii* ssp.	Ibb	13.12.2007
43	SBR 076	N41793	1	*H. yerburii* ssp.	Ibb	13.12.2007
44	SBR 077	N41794	1	*H. yerburii* ssp.	Ibb	19.11.2007
45	SBR 079	N41796	1	*H. yerburii* ssp.	Yareem	27.10.2007
46	SBR 087	N41797	1	*H. yerburii* ssp.	Ibb	20.01.2008
47	SBR 088	N41798	1	*H. yerburii* ssp.	Ibb	17.02.2008
48	SBR 089	N41799	1	*H. yerburii* ssp.	Ibb	17.02.2008
49	SBR 090	N41800	1	*H. yerburii* ssp.	Ibb	17.02.2008
50	SBR 091	N41801	1	*H. yerburii* ssp.	Ibb	24.11.2007
51	SBR 092	N41802	1	*H. yerburii* ssp.	Ibb	24.11.2007
52	SBR 093	N41803	1	*H. yerburii* ssp.	Ibb	25.11.2007
53	SBR 094	N41804	1	*H. yerburii* ssp.	Ibb	23.11.2007
54	SBR 095	N41805	1	*H. yerburii* ssp.	Ibb	23.11.2007
55	SBR 096	N41806	1	*H. yerburii* ssp.	Ibb	23.11.2007
56	SBR 097	N41807	1	*H. yerburii* ssp.	Ibb	25.11.2007
57	SBR 098	N41808	1	*H. yerburii* ssp.	Ibb	25.11.2007
58	SBR 099	N41809	1	*H. yerburii* ssp.	Ibb	19.11.2007
59	SBR 100	N41810	1	*H. yerburii* ssp.	Ibb	19.11.2007
60	SBR 101	N41811	1	*H. yerburii* ssp.	Ibb	20.11.2007
61	SBR 102	N41812	1	*H. yerburii* ssp.	Ibb	20.11.2007
62	SBR 103	N41813	1	*H. yerburii* ssp.	Ibb	20.11.2007
63	SBR 104	N41814	1	*H. yerburii* ssp.	Ibb	20.11.2007
64	SBR 105	N41815	1	*H. yerburii* ssp.	Ibb	26.11.2007
65	SBR 106	N41816	1	*H. yerburii* ssp.	Ibb	20.11.2007
66	SBR 107	N41817	1	*H. yerburii* ssp.	Ibb	28.11.2007
67	SBR 108	N41818	1	*H. yerburii* ssp.	Ibb	28.11.2007

Continuation of Appendix I.

68	SBR 109	N41819	1	*H. yerburii* ssp.	Ibb	01.12.2007
69	SBR 110	N41820	1	*H. yerburii* ssp.	Ibb	02.12.2007
70	SBR 111	N41821	1	*H. yerburii* ssp.	Ibb	06.12.2007
71	SBR 112	N41822	1	*H. yerburii* ssp.	Ibb	06.12.2007
72	SBR 113	N41823	1	*H. yerburii* ssp.	Ibb	07.12.2007
73	SBR 114	N41824	1	*H. yerburii* ssp.	Ibb	07.12.2007
74	SBR 116	N41826	1	*H. yerburii* ssp.	Ibb	05.01.2008
75	SBR 117	N41827	1	*H. yerburii* ssp.	Ibb	05.01.2008
76	SBR 118	N41828	1	*H. yerburii* ssp.	Ibb	05.01.2008
77	SBR 119	N41829	1	*H. yerburii* ssp.	Ibb	05.01.2008
78	SBR 123	N41830	1	*H. yerburii* ssp.	Ibb	06.01.2008
79	SBR 124	N41831	1	*H. yerburii* ssp.	Ibb	06.01.2008
80	SBR 125	N41832	1	*H. yerburii* ssp.	Ibb	06.01.2008
81	SBR 127	N41833	1	*H. yerburii* ssp.	Wadah	17.02.2008
82	SBR 128	N41834	1	*H. yerburii* ssp.	Wadah	17.02.2008
83	SBR 129	N41835	1	*H. yerburii* ssp.	Sanaa	26.02.2007
84	SBR 173	N41838	1	*H. yerburii* ssp.	Ibb	17.02.2009
85	SBR 176	N41840	1	*H. yerburii* ssp.	Ibb	17.02.2009
86	SBR 178	N41842	1	*H. yerburii* ssp.	Ibb	17.02.2009
87	SBR 180	N41844	1	*H. yerburii* ssp.	Ibb	18.02.2008
88	SBR 189	N41853	1	*H. yerburii* ssp.	Sanaa	10.02.2009
89	SBR 190	N41854	1	*H. yerburii* ssp.	Sanaa	10.02.2009
90	SBR 191	N41855	1	*H. yerburii* ssp.	Sanaa	10.02.2009
91	SBR 007	N41856	2	*H. y. yerburii*	Tour Albaha	22.08.2007
92	SBR 009	N41857	2	*H. y. yerburii*	Tour Albaha	17.11.2007
93	SBR 010	N41858	2	*H. y. yerburii*	Tour Albaha	17.11.2007
94	SBR 011	N41859	2	*H. y. yerburii*	Tour Albaha	03.01.2008
95	SBR 017	N41861	2	*H. y. yerburii*	Tour Albaha	20.11.2007
96	SBR 019	N41862	2	*H. y. yerburii*	Tour Albaha	22.08.2007
97	SBR 029	N41863	2	*H. y. yerburii*	Tour Albaha	10.11.2007
98	SBR 030	N41864	2	*H. y. yerburii*	Tour Albaha	15.11.2007
99	SBR 034	N41865	2	*H. y. yerburii*	Tour Albaha	03.12.2007

Continuation of Appendix I.

100	SBR 035	N41866	2	*H. y. yerburii*	Tour Albaha	01.12.2007
101	SBR 073	N41867	1	*H. yerburii* ssp.	Ibb	11.11.2007
102	SBR 120	N41868	2	*H. y. yerburii*	Tour Albaha	02.12.2007
103	SBR 121	N41869	2	*H. y. yerburii*	Tour Albaha	02.12.2007
104	SBR 122	N41870	2	*H. y. yerburii*	Tour Albaha	06.01.2008
105	SBR 137	N41871	2	*H. y. yerburii*	Radfan	11.03.2009
106	SBR 138	N41872	2	*H. y. yerburii*	Radfan	11.03.2009
107	SBR 140	N41873	2	*H. y. yerburii*	Shihr	02.03.2009
108	SBR 148	N41874	2	*H. y. yerburii*	Ariab	19.02.2009
109	SBR 149	N41875	2	*H. y. yerburii*	Ariab	19.02.2009
110	SBR 153	N41878	2	*H. y. yerburii*	Lowder	27.02.2009
111	SBR 156	N41880	2	*H. y. yerburii*	Lowder	27.02.2009
112	SBR 157	N41881	2	*H. y. yerburii*	Lowder	27.02.2009
113	SBR 159	N41883	2	*H. y. yerburii*	Lowder	01.03.2008
114	SBR 162	N41886	2	*H. y. yerburii*	Lowder	01.03.2008
115	SBR 167	N41887	2	*H. y. yerburii*	Aden	14.02.2009
116	SBR 175	N41888	2	*H. y. yerburii*	Tour Albaha	01.03.2009
117	SBR 014	N41889	3	*Hemidactylus* sp.	Ibb	17.11.2007
118	SBR 015	N41890	3	*Hemidactylus* sp.	Ibb	17.11.2007
119	SBR 018	N41891	3	*Hemidactylus* sp.	Ibb	15.11.2007
120	SBR 022	N41892	3	*Hemidactylus* sp.	Sana'a	28.11.2007
121	SBR 032	N41893	3	*Hemidactylus* sp.	Ibb	16.09.2007
122	SBR 033	N41894	3	*Hemidactylus* sp.	Ibb	30.11.2007
123	SBR 056	N41895	1	*H. yerburii* ssp.	Ibb	12.11.2007
124	SBR 057	N41896	1	*H. yerburii* ssp.	Ibb	09.11.2007
125	SBR 058	N41897	3	*Hemidactylus* sp.	Ibb	07.11.2007
126	SBR 070	N41898	1	*H. yerburii* ssp.	Thamar	24.11.2007
127	SBR 071	N41899	3	*Hemidactylus* sp.	Thamar	24.11.2007
128	SBR 192	N41900	3	*Hemidactylus* sp.	Sana'a	12.02.2009
129	SBR 193	N41901	3	*Hemidactylus* sp.	Sana'a	12.02.2009
130	SBR 083	N41902	4	*H. sinaitus*	Lahj	16.02.2008
131	SBR 085	N41903	4	*H. sinaitus*	Lahj	16.02.2008
132	SBR 163	N41904	4	*H. sinaitus*	Sh.Othman	21.03.2009
133	SBR 001	N41908	5	*Hemidactylus* sp.	Shihr	20.12.2007

Continuation of Appendix I.

134	SBR 006	N41909	5	*Hemidactylus* sp.	Shihr	13.10.2008
135	SBR 194	N41910	5	*Hemidactylus* sp.	Ghail Bawzeer	15.08.2008
136	SBR 195	N41911	5	*Hemidactylus* sp.	Ghail Bawzeer	15.08.2008
137	SBR 080	N41912	6	*Hemidactylus* sp.	Marib	27.10.2007
138	SBR 081	N41913	6	*Hemidactylus* sp.	Marib	27.10.2007
139	SBR 082	N41914	6	*Hemidactylus* sp.	Marib	10.02.2008
140	SBR 126	N41915	6	*Hemidactylus* sp.	Marib	10.02.2008
141	SBR 008	N41916	7	*Hemidactylus* sp.	Radman	15.11.2007
142	SBR 002	N41917	8	*Hemidactylus* sp.	Shihr	03.11.2007
143	SBR 003	N41918	8	*Hemidactylus* sp.	Shihr	28.09.2007
144	SBR 005	N41919	8	*Hemidactylus* sp.	Shihr	04.09.2007
145	SBR 012	N41920	8	*Hemidactylus* sp.	Shihr	03.01.2008
146	SBR 004	N42045	9	*Hemidactylus* sp.	Tebalah	04.09.2007
147	SBR 084	N42046	9	*H. flaviviridis*	Abyan	15.01.2008
148	SBR 086	N42047	9	*H flaviviridis*	Abyan	17.02.2008
149	SBR 136	N42048	9	*H. flaviviridis*	Radfan	11.03.2009
150	SBR 139	N42049	9	*H. flaviviridis*	Radfan	11.03.2009
151	SBR 142	N42050	9	*H. flaviviridis*	Shihr	02.03.2009
152	SBR 143	N42051	9	*H. flaviviridis*	Mukalla	03.03.2009
153	SBR 145	N42053	9	*H. flaviviridis*	Mukalla	03.03.2009
154	SBR 146	N42054	9	*H. flaviviridis*	Mukalla	03.03.2009
155	SBR 168	N42055	9	*H. flaviviridis*	Aden	14.02.2009
156	SBR 016	N42616	9	*H. flaviviridis*	Aden	13.10.2007
157	SBR 036	N42617	1	*H. yerburii* ssp.	Ibb	30.10.2007
158	SBR 115	N42618	1	*H. yerburii* ssp.	Ibb	07.12.2007
159	SBR 154	N42619	2	*H. y. yerburii*	Lowder	27.02.2009
160	SBR 196	N42620	5	*Hemidactylus* sp.	Ghail Bawzeer	15.08.2008
161	SBR 197	N42621	5	*Hemidactylus* sp.	Ghail Bawzeer	15.08.2008
162	SOQ1- 09	N29530	9	*H. flaviviridis*	Socotra	Feb. 1999
163	SOQ3- 12	N29533	-	*H. granti*	Socotra	Feb. 1999
164	SOQ3- 13	N29534	-	*H. granti*	Socotra	Feb. 1999
165	SOQ3- 14	N29535	-	*H. granti*	Socotra	Feb. 1999
166	SOQ3- 15	N29536	-	*H. granti*	Socotra	Feb. 1999
167	SOQ3- 16	N29537	-	*H. granti*	Socotra	Feb. 1999
168	SOQ7- 17	N29538	-	*H. homoeolepis*	Socotra	Feb. 1999

Continuation of Appendix I.

169	SOQ12- 20	N29541	-	*H. homoeolepis*	Socotra	Feb. 1999
170	SOQ31- 23	N29544	-	*H. homoeolepis*	Socotra	Feb. 1999
172	SOQ32- 24	N29545	-	*H. forbesii*	Socotra	Feb. 1999
173	SOQ32- 25	N29546	-	*H. oxyrhinus*	Socotra	Feb. 1999
174	SOQ32- 26	N29547	-	*H. forbesii*	Socotra	Feb. 1999
175	SOQ32- 27	N29548	-	*H. forbesii*	Socotra	Feb. 1999
176	SOQ32- 28	N29549	-	*H. forbesii*	Socotra	Feb. 1999
177	SOQ32- 29	N29550	-	*H. forbesii*	Socotra	Feb. 1999
178	SOQ32- 30	N29551	-	*H. forbesii*	Socotra	Feb. 1999
179	SOQ32- 32	N29552	-	*H. forbesii*	Socotra	Feb. 1999
180	SOQ32- 31	N29553	-	*H. oxyrhinus*	Socotra	Feb. 1999
181	SOQ37- 33	N29554	-	*H. granti*	Socotra	Feb. 1999
182	SOQ37- 34	N29555	-	*H. granti*	Socotra	Feb. 1999
183	SOQ37- 35	N29556	-	*H. granti*	Socotra	Feb. 1999
184	SOQ	N29592	-	*H. turcicus*	Socotra	Feb. 1999
185	SOQ3	N29593	-	*H. turcicus*	Socotra	Feb. 1999

Appendix II: the original measurements (in mm) of all meristic and morphometric characters of *Hemidactylus* specimens that were collected from the mainland (for the abbreviations, see table 7).

NHM-BS	*No.V.*	*No.D.*	*TD.*	*UL.*	*LL.*	*IntGra*	*Sc1stT*	*Sc4thT*	*MP*	*SVL*	*LH*	*WH*	*HH*	*Eye-D*	*Orbit-Ear*	*Orb-Sn*
N41751	36	83	16	11	8	1	7	10	11	51,4	16,5	11	6,2	3,4	4,6	6,2
N41753	40	76	14	11	8	1	6	10	10	57,3	17,3	10,8	6,8	3,1	5,2	7,4
N41755	41	90	14	9	7	1	6	10	8	40,9	14,4	9,6	5,4	3,8	4,2	6,2
N41756	42	86	16	9	8	1	6	10	-	45,2	15,4	9,7	5,6	3,2	4	6,2
N41758	38	84	16	10	7	1	6	10	-	64,1	19,3	12,9	7,5	4	5,1	7,3
N41760	40	97	16	10	8	1	6	10	-	41	16,6	11,1	6,7	3,6	4,6	6,9
N41761	43	89	16	11	9	1	6	10	9	50,5	18,1	12,7	6,8	3,4	4,4	6,5
N41762	45	89	16	11	9	1	6	10	-	50,2	15,9	9,8	6,8	3,3	4,7	6,5
N41763	48	94	16	12	8	1	6	10	-	52,3	17,6	11	6,4	3,5	4,2	6,4
N41764	41	86	14	10	9	1	6	10	10	43,4	14,4	10,1	6,8	3,2	4,2	6,3
N41765	44	77	16	10	8	1	6	10	-	53	17	11,4	6,4	3,8	4	6,4
N41766	40	87	16	11	8	1	7	11	-	47	14,8	10	6,4	3,2	4,6	6,2
N41768	48	74	14	11	8	1	7	11	-	48,3	15,4	10,7	6,4	3,3	4,8	7
N41769	43	96	16	10	7	1	6	10	12	47,6	17	11,2	7	3,8	5,3	7,3
N41770	45	79	14	10	8	1	6	11	9	52,3	17	11,6	6,8	3,9	5,4	7,4
N41771	44	97	14	11	7	1	6	11	-	57,1	19,3	11,9	6,3	3,6	4,6	7,1
N41772	38	81	14	10	8	1	6	10	11	52,8	17,5	12,8	7	4,1	5,3	7

Continuation of Appendix II.

N41773	46	81	15	11	8	1	6	10	-	46,8	15,7	10,5	6,3	3,3	4,2	6,3
N41774	48	74	16	11	8	1	7	11	12	43,8	16,3	13,1	5,7	3,4	4	6,6
N41775	36	89	14	10	9	1	7	11	-	32,5	13,1	7,8	4,6	3	3,2	5,4
N41776	42	74	14	11	8	1	6	11	-	39,7	14,3	8,3	4,8	3,1	3,5	5,3
N41777	44	76	16	10	8	1	6	10	-	34,2	11,2	7,2	4,5	2,7	3	4,4
N41779	35	64	12	9	8	1	6	9	-	46,5	14,4	10	6,2	3	4,7	5,5
N41780	33	77	12	11	9	0	6	11	-	37,1	14	8,4	4,2	2,7	4,4	5,1
N41781	36	89	16	9	8	1	6	10	8	45,5	13,6	10,2	5,6	3,1	4,2	5,9
N41783	40	91	15	10	8	1	6	10	11	48,5	16,2	12,3	7,7	3,6	5,3	6,5
N41784	44	76	16	11	9	1	7	10	-	43,2	16,9	11,3	7	4,1	4,9	6,7
N41785	38	88	14	10	7	1	6	10	12	67,7	19,2	13,6	6,8	4,2	4,8	7,3
N41786	45	78	16	10	7	1	6	10	-	51,2	16,7	10,1	5,9	3,5	4,1	6,9
N41787	42	91	14	10	8	1	6	9	-	39,1	12,3	7,7	4,5	3,2	3,4	5,3
N41788	38	86	14	10	8	1	6	10	7	50,5	16,6	10,6	6,4	3,3	4,4	6,2
N41791	36	88	16	10	8	1	7	11	9	46,3	17,3	11,7	6,6	3,8	4,9	6,8
N41793	36	94	16	10	7	1	7	10	11	52,3	17	11,5	7	4,3	4,8	6,9
N41794	39	79	16	10	7	1	6	10	-	39	13	8,6	5,2	3,8	3,8	5,3
N41795	40	81	14	10	8	1	7	11	-	57,4	17,7	11,9	5,8	4,3	4,7	6,9
N41796	43	83	14	10	7	1	7	11	10	39,2	13,4	8,6	5,2	3,1	3,6	5
N41797	42	94	16	10	8	1	6	10	10	47,7	15,6	10,6	6,3	3,5	4,4	6,3
N41798	40	88	16	10	8	1	6	10	10	43,8	14,8	10	5,8	3,1	4,2	6,2
N41799	44	82	16	11	8	1	6	11	-	47,3	15	10,9	6,6	3,3	4,6	6,3

Continuation of Appendix II.

N41800	48	93	14	10	8	1	6	10	10	55,9	16,2	12,4	7,3	3,9	5,5	7,3
N41801	36	84	14	10	8	1	6	9	-	23,5	8,4	5,1	3,7	2	2,3	2,9
N41802	44	88	15	10	7	1	6	10	8	61,8	18,8	14	8,7	4,4	6,2	7,8
N41803	48	87	16	11	8	1	6	10	11	54	16,7	11,8	7,2	3,1	5	7,4
N41804	48	80	14	10	8	1	7	10	11	54,2	17,2	12,1	7,3	3,5	4,8	5,9
N41805	36	93	13	10	8	1	6	10	10	60,9	18,6	11,8	7,9	3,8	4,5	7,9
N41806	47	90	16	11	8	1	6	11	-	47,5	16,3	10,6	7,1	3,5	4,8	6,6
N41807	38	91	14	11	9	1	6	10	10	52,2	18,1	11,8	6,9	4,1	5	6,7
N41808	39	85	16	10	8	1	6	9	-	31	10,8	6,7	4,3	2,4	2,9	3,8
N41809	42	76	16	10	8	1	6	9	-	60,5	18,8	11,6	7,4	4,3	4,6	7
N41810	41	79	16	10	8	1	6	10	-	32,3	10,7	6,4	4,7	2,5	2,8	3,5
N41811	42	80	16	10	7	1	6	11	-	59,4	17,7	12,3	7,3	4,1	5,1	7,4
N41812	43	81	16	11	9	1	6	10	10	54,7	19,8	13,1	7,9	4	5	7,6
N41813	43	85	16	10	7	1	6	10	-	47,3	15,2	10,5	6,5	3,3	4,8	6,7
N41814	39	97	16	10	7	1	7	10	12	56,7	19,3	11,4	7,6	4,1	5,5	7,9
N41815	38	80	16	11	7	1	7	11	-	46,6	13,8	9,1	5,2	3,3	3,8	7,9
N41816	46	81	16	12	8	1	6	10	-	31,6	10,7	6,9	4,8	2,4	3,5	6
N41817	37	89	16	10	8	1	7	11	-	32,8	12,7	8,1	5,3	3,5	4,9	5,4
N41818	43	96	15	9	7	1	5	10	-	37,8	13,4	9,5	5,7	3	4,2	5,5
N41819	38	91	16	10	8	1	6	10	-	43,4	14,7	10	5,6	3,6	3,9	5,3
N41821	47	74	16	11	8	1	6	10	-	55	16	10,8	6,5	3,6	4,3	6,6
N41822	43	79	16	10	7	1	7	10	-	43,9	14,3	10	6	3,5	4,6	6

Continuation of Appendix II.

N41823	38	97	16	10	8	1	7	10	-	51,3	15,1	10,6	5,5	3,8	3,8	6,4
N41824	40	94	16	10	8	1	6	11	-	43	13,8	9,3	5,6	3,3	3,6	5,6
N41825	46	93	14	10	7	1	6	10	-	45,1	13,7	9,7	6,3	3,2	3,5	5,8
N41826	38	97	16	9	8	1	6	10	-	37,5	12,3	8,5	5,1	3	3,5	4,8
N41827	48	93	14	11	8	1	6	10	-	35,1	11,6	7,6	4,6	3	3,4	4,9
N41828	43	89	16	10	8	1	6	9	-	30,5	10,9	6,8	3,8	2,5	2,9	4,1
N41829	36	92	16	10	7	1	5	10	-	37,3	12,3	8,9	6,3	3	3,4	4,6
N41830	44	92	16	10	8	1	7	10	-	22,9	8,6	4,9	2,8	2,3	2,3	3,3
N41831	42	96	14	11	8	1	6	10	-	33,6	11	6,9	4,8	3	3,3	4,6
N41832	48	84	16	10	8	1	6	10	-	36,3	11,6	7,5	4,8	2,6	3	4,3
N41833	44	90	14	10	8	1	6	9	-	49,8	15,3	10,1	6,5	3,9	4	6,2
N41834	46	78	14	10	7	1	6	9	-	54,6	16,3	11,5	6,6	4	4,3	6,3
N41836	44	85	16	10	8	1	7	10	-	49,1	16,1	10,8	6,5	3,8	5	7,1
N41837	48	91	16	10	8	1	6	10	-	30,5	9,9	6,7	4	2,6	2,9	4,3
N41838	43	83	16	11	7	1	7	10	-	46,4	13,3	10,1	5,5	3,2	3,7	5,4
N41839	42	96	14	10	8	1	7	10	-	48,8	15	10,4	6,4	3,3	4,3	6,2
N41840	37	92	16	11	8	1	7	10	-	51,3	16	10,4	7	3,3	3,8	5,8
N41841	42	86	16	10	9	1	6	10	-	38,3	12,4	8,5	5	3,4	3,9	5,4
N41842	36	85	16	11	8	1	6	10	12	43	13,8	9	5,1	3,1	3,7	5,8
N41843	43	89	16	11	8	1	6	10	12	52,3	16,3	12	6,4	3,5	5,5	7,2
N41844	42	86	16	10	8	1	6	10	10	42,4	13,5	9,5	6,5	3,1	4,1	5,8
N41845	39	82	16	11	8	1	7	10	-	42	14	8,6	6	3,4	3,9	5,6

Continuation of Appendix II.

N41846	46	74	16	11	8	1	6	10	-	46,5	15,5	10,5	6,6	3,9	4,2	6,4
N41847	41	97	16	10	8	1	7	10	-	40	13,4	9,2	5,7	3,1	3,9	5,7
N41848	36	84	14	11	8	1	6	10	9	53,6	17,4	12,2	6,9	3,6	5	7,3
N41849	46	83	16	10	7	1	6	10	-	27,3	9,4	6,2	3,9	2,3	2,8	3,6
N41850	44	82	16	11	8	1	6	10	-	30,3	10,4	6,4	4,2	2,4	3	4,4
N41851	44	92	16	10	8	1	6	11	10	49,9	15,8	11,6	6	3,4	4,8	6,7
N41852	40	89	16	12	9	1	7	11	11	60,1	18,8	12,7	7,4	4,2	5,2	7,4
N41853	41	77	14	11	8	1	6	10	-	47,3	15	9,9	6,1	3,4	4,4	6,3
N41854	47	77	16	10	8	1	7	11	-	49	15,2	9,7	5,5	3,2	4,1	6
N41855	38	76	14	11	8	1	6	10	-	39,5	12,8	7,3	4,1	3,1	3,3	5
N41856	45	98	14	10	7	1	6	10	-	58,4	18,3	11,9	7,2	3,8	5	8
N41857	46	102	14	11	7	1	7	10	-	50,2	17,3	11	7,4	3,8	4,4	7,3
N41858	46	86	14	11	7	1	7	10	13	47,3	15,2	11,1	7,2	3,6	4,3	6,6
N41859	47	99	14	10	7	1	6	10	17	57,2	20	14,1	8,4	4,2	5	7,2
N41860	47	98	14	10	9	1	7	10	-	42,9	14,5	9,1	5,9	3,5	4,4	6,3
N41861	43	98	14	10	7	1	7	11	-	61,5	18,7	12,8	8,1	4	5	6,9
N41862	43	95	16	11	8	1	6	10	14	54,1	18,7	13,1	8,2	3,7	5	6,8
N41863	42	97	16	10	8	1	7	10	-	55,5	16,7	12,3	7,3	4,2	4,3	7,7
N41864	44	86	16	10	7	1	6	10	-	41,4	15,7	10,1	6,9	4,2	5,1	7,8
N41865	37	97	14	12	7	1	6	11	-	40,6	13,9	9,9	6	4	4,6	7,2
N41866	41	91	16	10	8	1	6	11	-	49,8	17	10,9	7,1	4	4,7	7,4
N41867	43	87	16	11	8	1	6	10	-	60	18,8	11,1	8,2	4,4	4,5	7,7

Continuation of Appendix II.

N41868	42	98	16	11	8	1	7	10	12	64,3	21,3	15,6	9,5	4,4	6,3	9,2
N41869	34	92	16	12	9	1	7	10	-	42,6	13,4	8,4	5,6	3,1	3,6	5,4
N41870	38	82	14	10	8	1	7	10	-	35,8	12	7,5	4,7	3	3,4	4,6
N41871	40	92	16	12	9	0	7	10	-	46,1	14,6	8,9	6,2	3,5	4	6,4
N41872	42	84	16	12	8	0	7	11	-	54,1	16,9	10,8	6,9	3,8	4,4	7,4
N41873	50	91	16	10	8	1	7	11	13	54,6	18,3	12,1	7,9	4	4,7	7,7
N41874	44	84	16	10	8	1	7	10	-	35,3	11,7	7,4	4,8	2,7	3,3	5
N41875	38	83	16	10	8	1	7	10	-	53,7	15,7	11,3	6,7	3,9	4,7	6,7
N41876	32	91	14	11	8	1	7	11	11	53,5	17,3	11,8	7,1	4	5	7
N41877	46	89	16	10	8	1	7	11	-	58,5	16,5	11,5	7	4	5	7,8
N41878	34	88	14	10	8	1	7	10	-	37,1	13	8,1	4,5	2,9	3,4	5,4
N41879	42	98	16	11	8	1	7	11	-	49,9	16	10,4	6,7	4,1	4,7	7
N41880	46	84	16	10	8	1	7	11	-	58,8	17,4	11,2	7,1	4,3	4,6	7,4
N41881	36	90	16	10	8	1	7	10	11	63,6	19,7	13,2	8,1	3,8	4,6	8,5
N41882	36	86	16	10	8	1	7	10	-	55,4	18,1	12,4	6,9	3,6	4,8	8
N41883	34	97	16	11	8	1	7	10	-	54,1	16,1	10,8	6,4	3,6	4,5	7,3
N41884	46	86	16	10	8	1	7	10	-	58,7	17,4	11,2	7	3,6	4,5	7,3
N41885	40	88	16	10	8	1	7	10	10	48,2	16,1	10,8	6,2	3,6	4,4	7
N41886	38	94	16	10	8	1	7	10	-	59,9	18,6	12,5	7,1	3,9	5	7,8
N41887	38	92	16	10	8	1	6	10	12	52,7	15,6	10,8	6,4	3,7	4,8	7
N41888	36	87	16	10	8	1	7	11	12	64,4	20,1	13,4	7,8	4,2	5,7	8,3
N41889	36	68	12	10	8	1	6	9	-	28,6	9,9	5,9	3,3	2,5	3,3	4

Continuation of Appendix II.

N41890	35	68	12	11	8	1	6	11	-	46,4	14,7	9,7	5,2	3,4	4,6	6,2
N41891	34	65	12	10	8	0	7	11	6	45,1	15,6	11,1	5,4	3,1	4,4	6,4
N41892	33	66	12	10	9	0	6	10	-	37,9	12,2	8,1	4	2,8	3,7	4,7
N41893	36	62	10	10	9	1	6	10	-	47	14,7	10,3	5,7	3,6	4,9	5,9
N41894	42	66	12	9	8	1	7	11	9	45,7	15,9	10,2	5,3	3,5	4,3	5,8
N41897	36	62	12	9	7	1	7	10	-	47,6	15,5	10,5	6,6	3,3	4,8	6,1
N41899	38	76	13	10	8	1	7	10	-	44,6	15,6	9,6	5,2	3,1	4,4	5,3
N41900	34	62	14	10	8	1	7	11	-	35,9	11,8	7,1	3,8	3	3,7	4,7
N41901	33	65	14	10	8	0	6	10	-	38,7	13,4	8,9	5,6	3	3,9	4,8
N41902	38	71	15	9	7	1	5	9	-	38,7	11,2	7,2	5,3	2,3	3	3,6
N41903	38	70	14	8	7	1	5	9	-	32,4	10	6	4,3	2,8	3,7	4,5
N41904	34	68	14	8	8	1	5	10	7	37	10,5	6,8	5,1	2,5	3,4	4,2
N41905	32	75	14	9	7	1	5	9	-	29,2	9,2	5,8	3,8	2	2,7	3,4
N41906	34	77	14	9	7	1	5	9	-	24,4	8,3	4,9	3,3	2,1	2,5	3,4
N41907	33	72	14	9	7	1	5	9	-	22,5	8,8	5,2	3,4	2,4	2,8	3,6
N41908	49	71	14	9	7	1	6	10	-	38,9	13	8	5,5	3,4	3,2	5,6
N41909	46	69	14	10	8	1	6	10	-	29	10,7	6	4,3	2,7	2,6	4,5
N41910	51	82	12	9	8	1	6	10	6	53	17,3	10,8	6,3	3,2	4,2	6,6
N41911	54	74	14	10	8	1	6	10	6	48,2	15,6	10	5,7	3,3	4,3	6,4
N41912	30	77	14	9	8	1	8	11	6	56,1	16,2	11,4	6,6	3,3	4,7	7,2
N41913	32	82	14	9	8	1	8	11	-	50,7	14,1	8,4	5,8	3,2	4,4	5,1
N41914	28	78	14	9	8	1	8	11	-	59,4	16,2	9,8	5,7	3,8	4,8	6,3

Continuation of Appendix II.

N41915	32	76	14	8	8	1	8	11	6	43	13,5	8,3	5,6	3,5	4,5	5,5
N41916	41	63	14	8	8	1	5	8	-	31,3	10,4	6,2	3,7	2,8	2,8	4
N41917	36	61	16	8	7	1	6	9	-	25,9	6,8	4,9	3,3	2,1	2,8	3,6
N41918	37	74	16	9	7	0	6	10	-	48	12,5	7,8	4,9	3,1	3,6	5
N41919	39	75	14	8	7	1	6	10	-	43,8	12,7	7,9	5,5	3	3,5	5
N41920	39	70	16	9	8	1	6	10	-	35,5	10,2	6,6	4,7	2,6	2,8	4
N42044	38	74	16	9	8	1	7	10	-	38,6	12,2	7,1	5	2,6	3,8	4,6
N42045	36	88	0	11	9	1	8	12	-	38,8	12,8	7,4	5	3,4	3,6	4
N42046	37	85	0	14	11	1	9	12	-	64,9	18,6	12,8	8,3	4,6	5,6	8,4
N42047	38	92	0	11	10	1	9	14	-	65,7	19,5	12,7	8,3	4	5,3	8,1
N42048	37	91	0	12	10	1	9	13	-	50,4	14,8	10,1	6,5	3,8	4,4	7,3
N42049	42	97	0	11	10	1	9	13	10	73,9	20,8	14,1	8,5	4,4	6,3	9,3
N42050	40	95	0	14	11	1	8	12	-	57,6	17,5	11,9	7,6	4	5,2	7,7
N42051	38	86	0	11	10	1	8	12	14	58,1	18	12,1	6,6	4,2	5,4	7,4
N42052	40	91	0	13	10	1	8	13	-	67,7	19,3	13,5	8,4	4,3	5,8	8,6
N42053	43	97	0	14	10	1	8	12	-	65,3	19,9	13,8	8,6	4,1	5,3	8,1
N42054	38	84	0	14	10	1	8	12	-	54,9	16,5	10,7	7	3,6	4,6	7,3
N42055	41	87	0	13	10	1	9	12	-	41,7	13	8,1	5,1	3,1	4	5,3
N42056	39	96	0	12	10	1	9	12	-	70,5	17,6	13,5	8,5	4,5	5,4	9,2
N42057	36	89	0	13	10	1	8	12	16	58,2	18	12	6,8	4	5,4	7,3
N42058	40	91	0	13	11	1	9	13	11	66	20,7	14,9	9,1	4,1	6	9,7
N42059	39	94	0	13	10	1	9	13	14	62,2	17,2	12,6	8,2	4	5	7,6